DISSERTATIONES SCHOLAE DOCTORALIS AD SANITATEM INVESTIGANDAM
UNIVERSITATIS HELSINKIENSIS

RITVA LINDBLAD

SEALING AND POST SYSTEMS´ ADHESION TO ROOT CANAL AFTER DENTINE IRRIGATION WITH CHLORHEXIDINE AND DIMETHYL SULFOXIDE

DEPARTMENT OF ORAL AND MAXILLOFACIAL DISEASES
FACULTY OF MEDICINE
DOCTORAL PROGRAMME IN ORAL SCIENCES
UNIVERSITY OF HELSINKI

Department of Oral and Maxillofacial Diseases
Faculty of Medicine
University of Helsinki, Finland

SEALING AND POST SYSTEMS´ ADHESION TO ROOT CANAL AFTER DENTINE IRRIGATION WITH CHLORHEXIDINE AND DIMETHYL SULFOXIDE

Ritva Lindblad

DOCTORAL DISSERTATION
To be presented for public discussion with the permission of the Faculty of Medicine, University of Helsinki in auditorium 2 of the Institute of Dentistry,

Mannerheimintie 172, Helsinki, on 31st of January 2020, at 12 noon.

Helsinki 2020

Supervised by Professor Leo Tjäderhane DDS, PhD
Department of Oral and Maxillofacial Diseases
University of Helsinki,
FINLAND
and
Professor Pekka Vallittu DDS, PhD, CDT
Department of Biomaterials Science and Turku Clinical
Biomaterials Centre-TCBC,
Institute of Dentistry
University of Turku,
FINLAND

Reviewed by Senior Lecturer Josette Camilleri,
School of Dentistry,
Institute of Clinical Sciences,
College of Medical and Dental Science
University of Birmingham,
UNITED KINGDOM
and
Professor Luc van der Sluis
Faculty of Medical Sciences
University of Groningen,
THE NETHERLANDS

Opponent Associate Professor Hagay Shemesh
Faculty of Dentistry ACTA
University of Amsterdam
THE NETHERLANDS

ISBN 978-951-51-5769-0 (nid.)
ISBN 978-951-51-5770-6 (PDF)

Hansaprint Oy

Helsinki 2020

The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations.

SEALING AND POST SYSTEMS´ ADHESION TO ROOT CANAL AFTER DENTINE IRRIGATION WITH CHLORHEXIDINE AND DIMETHYL SULFOXIDE

CONTENTS

ACKNOWLEDGEMENTS

This study was carried out at the department of Oral and Maxillofacial diseases, University of Finland and Department of Biomaterials Science and Turku Clinical Biomaterials Centre-TCBC, Institute of Dentistry, University of Turku, Turku, Finland and City of Turku, Welfare Division during the years 2010-2019.

My deepest gratitude goes to my supervisors Professor Leo Tjäderhane and Professor Pekka Vallittu. Professor Tjäderhane suggested this subject for me years ago and without hesitation offered to guide me with the research. He has been guiding me and mentoring me all these years. I am also deeply grateful to Professor Pekka Vallittu for his assistance and possibility to use the equipment's of his excellent laboratory in Turku Clinical Biomaterials Centre-TCBC. I feel privileged to have been able to work with these intelligent and kind persons.

I express my gratitude to DDS Lippo Lassila, who with warm heart guided me to use the testing equipment's and analyse all the curves and microscopic views.

I warmly thank the staff of the Biomedicum laboratory for technical help. Kirsti Kari as the chief of the laboratory, who always made space for me when I needed to use the laboratory. Jukka Inkeri, who came from his days of retirement and helped me to build up the fluidfiltration equipment and Ritva Keva and other staff of the laboratory, who took care of my specimen every day in the laboratory.

I also warmly thank DDS Veijo Salo for assisting me with the measurements and fracture mode analysis.

I am very grateful for Student Health Care Organization of Tampere, Oulu and Helsinki for the supply of extracted third molars. Especially Magda Marinescu-Gava, who collected the greatest amount of third molars for my research.

I also thank the importers and manufacturers for providing the materials for this research. Special thanks to Peter Åhren (Ultradent), Artti Juusela (3M), Hannele Nokia (Hammasväline) and Teemu Visuri (SybronEndo).

I warmly thank my follow-up group professor Pentti Kemppainen, DDS Kaija Hiltunen and DDS Anja Kotiranta, who always spurred me with my work. Especially Kaija and Anja I am grateful for the long telephone discussions whenever I needed support and good advice along the way.

Many dear colleges have been invaluable support for my work. They have listened me and my problems and helped me to clarify my ideas. Many thanks to Tanja Ketola-Kinnula, Tiiju Vuorjoki-

Ranta, Päivi Siukosaari, Annamari Nihtilä, Terhi Karaharju-Suvanto, Johanna Höök-Nikanne and many other colleagues.

I am grateful to the financial support of the Finnish Dental Society Apollonia and Finnish Women Dentists’ Association for supporting work.

I owe my dearest thanks to my daughters Maria and Kristina and my husband Markku. You always had faith in me and supported me with my work. Without you and your love this would not have happened. I love you.

Helsinki, 2019

ABREVIATIONS

AD	adhesive-to-dentin fracture mode
AP	adhesive-to-post fracture mode
BAC	benzalkonium chloride
BPDM	Bisco's own patented hydrophilic monomer
BIS-GMA	bisphenol-A-glysidyl methacrylate
BPO	benzoyl peroxide
C	cystein
Cat	catalytic domain
CC	cohesive-in-cement fracture mode
CD	cohesive-to-dentin fracture mode
CHX	chlorhexidine
CSC	calcium silicate cement
CTs	cysteine cathepsins
CTR	control
DMSO	dimethyl sulfoxide
EDTA	ethylenediaminetetraacetic acid
FRC	fiber reinforced composite
hCSC	hydraulic calcium silicate cement
HEMA	2-hydroxyethyl methacrylate
Hpx	hemopexin
IPN	interpenetrating polymer network
MDP-10	methacryloxy-decyl-dihydrogen-phosphate
4-META	4-methacryloxyethyl trimellitate anhydride
MMP	matrix metalloproteinase enzyme
MPa	megapascal
MTA	mineral trioxide aggregate
N	newton
NaOCl	sodium hypochlorite
NiTi	nickel titanium

nl	nanoliter
NTG-GMA	N-tolylglycine glycidyl methacrylate
PMMA	polymethylmethacrylate
PRO	prodomain
SD	standard deviation
Semi-IPN	semi-interpenetrating polymer network
TEGDMA	triethylene glycol dimethacrylate
TIMP	tissue inhibitors of metalloproteinases
UDMA	urethane dimethacrylate
μl	microliter
VH	Vickers hardness

ABSTRACT

Dental caries or trauma may cause loss of coronal material so that endodontic treatment is needed. The aim is then to avoid or eliminate the infection, to replace the lost tissue and to restore the function and esthetics of the dentition. A tight and durable interface between the material in root canal and root dentin is crucial to inhibit the bacterial reinfection and to assure the longevity of the restauration. The bond strength of composite resin restoration on coronal dentin decrease with time. Previous studies have shown that chlorhexidine (CHX) and dimethyl sulfoxide (DMSO) preserve the long term bond strength between the restauration and coronal dentin. They both act as matrix metalloproteinase (MMP) -enzyme inhibitors which may be one reason to the bond strength preserving ability. DMSO has also the ability to reduce dentin surface free energy, improve wettability (slightly also CHX) and by that to enhance the adhesive penetration. The information of the effect of CHX and especially of DMSO on the adhesion of root canal sealers and calcium silicate cements (CSCs) to root canal dentin is scarce.

This series of studies investigated the effect of CHX and DMSO on the immediate and long term bond strength of chemically different resin cements, sealers and CSCs. If the bond strength of resin cements, sealers and CSCs could be improved and preserved by using MMP inhibitors, it would have a great importance to the restorative dentistry. By improving the adhesion the risk of bacterial penetration in the bonding interface decreases, the longevity of the root filling and restoration with root canal post is improved, the fracture risk of teeth restored with fiber posts decreases, and the need for retreatment is also decreased.

Intact third molars extracted as a part of normal treatment were used in all studies with the patients' consent and approval from the Ethical Committee, Faculty of Medicine, University of Oulu. The root canals were prepared by the normal endodontic protocol and, depending on the study, filled with different tested materials. In studies I and II four different fiber-reinforced composite posts were cemented with three different composite luting cements. In study III the root canals were obturated with gutta-percha and two different sealers and in study IV with two different CSCs. In every study the test groups was irrigated with CHX. In studies III and IV the test groups were irrigated either with CHX or DMSO. The controls were irrigated with sterile saline in every study. The measurements were done both immediately and after six, 12 or 18 months, depending on the study. In studies I, II and IV the bond strength was measured with push-out method and the fracture mode was analyzed with stereomicroscope. The sealing ability in study III and IV was tested also

with fluid filtration method. In study IV the effect of CSCs on dentin biomineralization was also evaluated by using Vickers hardness test after aging.

2% CHX did not affect the immediate bond strength of the root canal post/composite cement to root dentin, but improved the long-term bond strength of one (out of four) post/cement combination. Neither CHX nor DMSO showed statistically significant differences in the immediate microleakage within two tested sealers or with CSCs. However, final irrigation with DMSO caused significantly less microleakage than CHX irrigation for both tested sealers in 18 months aged samples ($p<0.01$ for Topseal and $p<0.05$ for RealSeal SE; Mann-Whitney test). With CSCs no effect on the long-term microleakage was seen with either CHX or DMSO. Dentin hardness was significantly higher in ProRoot MTA-control and ProRoot MTA-DMSO groups than in Biodentine groups (Kruskal-Wallis and Mann-Whitney tests, $p<0.05$), indicating that Pro-Root MTA's ability to increase biomineralization is better than that of Biodentine's.

As conclusion 2% CHX may at least moderately improve the long-term post adhesion, but the effect may be material dependent. On the other hand 2% CHX may negatively affect the bond strength of ProRoot MTA and the failure rate of both CSCs. DMSO as a final irrigant resulted with the lowest long-term leakage with both sealers and with Biodentine and the lowest adhesive failure rates with both CSCs, indicating that probably the superior wettability induced by DMSO contributes to this interface integrity. Although the amount of leakage in these studies (III and IV) was very small, it cannot assume that these obturations with sealers or with CSCs would have totally inhibited the bacterial invasion with time. In addition, considering that aging increased the leakage in all CSC groups except with Biodentine-DMSO and the differences in the push-out strength and Vickers hardness data, it appears that the time-related biomineralizing effect of MTA and Biodentine does not improve sealing or bonding to dentin.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications

I. **Lindblad RM**, Lassila LV, Salo V, Vallittu PK, Tjäderhane L. Effect of chlorhexidine on initial adhesion of fiber-reinforced post to root canal. Journal of Dentistry 2010;38(10): 796-801.

II. **Lindblad RM**, Lassila LV, Salo V, Vallittu PK, Tjäderhane L. One year effect of chlorhexidine on bonding of fibre-reinforced composite root canal post to dentine. Journal of Dentistry 2012;40(9):718-722.

III. **Lindblad RM**, Lassila LV, Vallittu PK, Tjäderhane L. The effect of chlorhexidine and dimethyl sulfoxide on long-term microleakage of two different sealers in root canals. European Endodontic Journal 2019:4(1):38-44

IV. **Lindblad RM**, Lassila LV, Vallittu PK, Tjäderhane L. The effect of chlorhexidine and dimethyl sulfoxide on the immediate and long-term sealing ability of two different calcium silicate cements. Submitted

1. INTRODUCTION

In dentistry the researchers have for decades tried to create an impermeable adhesion between different chemically compound materials and tooth tissue and it still is one of the most important issues in restorative dentistry. The requirements for the dental materials are high. The materials and especially the adhesion to dentin and enamel should withstand the masticatory forces, material dependent degradation, enzymatic hydrolysis and thermal changes, not to mention the patient related factors such as cleaning and eating habits etc.

In dentin bonding the adhesive penetrates between the exposed collagen network forming the hybrid layer, the base of adhesive bonding (Breschi et al. 2018). Some moisture is needed to keep the collagen network open to allow the adhesive penetration between the collagen fibers. Another mechanism involving the dentinal bonding is the penetration of adhesive into the dentinal tubules (Maroulakos et al. 2018). Bonding to radicular dentin differs from bonding to coronal dentin in several ways (Maroulakos et al. 2018). The root canal is a deep and downwards narrowing space, where the moisture control along the canal may be difficult. The amount and diameter of dentinal tubules decrease towards the apical part of the root (Pashley et al. 1995) and the constant light penetration may be difficult to achieve causing incomplete polymerization of the adhesive or resin-based material (Wu et al. 2009).

Great efforts have been made to find techniques and materials to increase the durability of the adhesion and sealing ability. One main reason to jeopardize dentin bonding is the degradation of collagen in the hybrid layer. The collagen degradation may be caused by enzymatic function. Matrix metalloproteinases and cysteine cathepsins both degrade the collagen matrix in hybrid layer (Breschi et al. 2018). These enzymes need water trapped in the hybrid layer to function and to degrade the collagen by hydrolysis.

This series of studies concentrated to the effect of two different irrigants, CHX and DMSO, on bonding and sealing of root canals with different obturation materials. CHX is commonly used as an irrigant in endodontic treatment due to its good antimicrobial properties and adhesion into root canal dentin (Basrani et al, 2002, Basrani and Lemonie 2005). DMSO is a solvent that has long history in industry and pharmacology and it has been used as a penetration enhancer for medical compounds since 1960 (Marren et al. 2011). Both CHX and DMSO have inhibitory effect on collagen degradative enzymes (Gendron et al. 1999, Tjäderhane et al. 2013a). In addition, DMSO increases dentin wettability (Mehtala et al. 2017, Stape et al 2015), which may improve and preserve the adhesion of restorative composite resins to coronal dentin (Stape et al. 2016a, Stape et al. 2015, Tjaderhane et al. 2013b).

The root canal obturation and restorative materials used with the tested irrigants in all our studies are commonly used in endodontic and restorative treatments of root canal-treated teeth. Fiber reinforced composite posts cemented with resin cement are used to anchor coronal restorations into the root, endodontic sealers are used to seal the interface between the gutta-percha and dentin in root canal obturations, and calcium silicate cements are used for example to close open root canal apices or to repair root canal perforations.

The primary specific questions in the four studies of this thesis were:

- What is the effect of CHX on immediate or long-term attachment of fiber posts cemented into root canals with resin luting cements
- What is the effect of CHX and DMSO on the immediate and long-term sealing ability and adhesion of root canal sealers and CSCs
- What is the potential effect of CHX and DMSO on the CSC-induced biomineralization of root canal dentin

There is a clear need in restorative dentistry to develop materials that allow reproducable creation of impermeable root canal obturations and economical restorations. Better understanding of the effect of CHX, which already is in common use in endodontics, and DMSO, which could have a wider use in dentistry, might result with more durable adhesion of materials already in clinical use. In addition the need for retreatments caused by material failure could diminish.

It is always a challenge to balance between the results of in vitro studies and their interpretations and relation to clinical protocols. While clinical protocols need always to be based on the in vitro results, they should always be a conclusion of results with different methods and has to be made with caution. The measurements in these studies were made with different testing methods commonly used in endodontic research. Bond strength was measured with push-out test, leakage with fluid filtration test and dentin hardness with Vickers hardness test. They all provide information of the materials and their behavior and effect on adhesion in laboratory environment from a different point of view, thus offering a more comprehensive view and subsequently increasing the clinical relevance and reliability.

2. REVIEW OF THE LITERATURE

2.1 Materials used in root canal obturation

Several centuries ago gutta-percha (then known as Mazer wood) was brought to England but not until 1840´s it has been used for many different products in Europe. From 1840´s gutta-percha, polymer of isoprene, has also been known to dentistry and Dr Bowman is said to have used gutta-percha as root canal filling for the first time in 1867 (Goodman et al. 1974, Briseno Marroquin et al. 2015). After the first root fillings with gutta-percha, many different obturation techniques have been used in endodontics, for example single-cone, master cone with accessory cones, warm vertical condensation and cold lateral condensation techniques (Whitworth 2005). 1978 Johnson introduced a new thermoplastic gutta-percha filling technique, where the instrument is coated with gutta-percha by warming it in a flame (Johnson 1978). After that more advanced warm gutta-percha devices has come to the market with specific heating systems. Despite several attempts to find alternative root canal obturation materials, gutta-percha itself has held its place in endodontics during centuries, but it always needs a sealer to fix and tighten the root filling.

2.1.1 Root canal sealers

Important factors in endodontic success are cleaning the bacterial debris, disinfection of the canal and a good obturation. In tight obturation the quality and mechanical properties of the sealing material play a crucial role in addition to the endodontic cone. On the other hand it has been stated that apical periodontitis can be healed without obturation if the shaping and cleaning has been done by the book and there is an absolutely tight coronal seal (Sabeti et al. 2006). It is nearly impossible to get perfectly tight seal with any root canal filling material with all possible variations of the root canal system. Although the contemporary antimicrobial irrigating agents and systems are very effective, one cannot be absolutely sure the root canal system is free from microorganisms capable to cause reinfection. The root canal obturation can however reduce the space and nutrition of these microbes and possibly prevent their multiplication (Sabeti et al. 2006).

Sealer is a material used to fill up the space between the gutta-percha and the canal walls. It ought to fill out the whole space of the challenging root canal system, possible lateral canals and other irregularities of the canal system. Ideally, the sealer should also penetrate dentinal tubules. Many different kind of sealers are nowadays on the market, the most popular being the epoxy resin-based sealers and methacrylate resin-based sealers. These sealers have been developed based on the chemistry used in adhesive restorative dentistry to get the adhesive character to the obturation technic.

Epoxy resin based sealers

Epoxy resin-based materials, such as TOPSEAL / AH Plus (Dentsply Maillefer, Ballaigues, Switzerland), are based on epoxy-amine polymer. According to the manufacturer these materials are chemically inert after curing. On the other hand these hydrophilic sealers have a tendency to expansion over time caused by water sorption from the dentinal tubules. This phenomena reduces the shrinkage stress in the root canal (Örstavik et al. 2001, Carvalho et al. 2007). The amount of shrinkage is an important factor because even 1% shrinkage can cause gap formation between the sealer and dentin or gutta-percha. The gaps may be large enough to allow bacterial penetration (Örstavik et al. 2001) especially if they link to each other. Intracanal gaps are shown to be fewer and smaller when the canal is obturated with gutta-percha and epoxy resin-based sealer (De-Deus et al 2011, Hammad et al. 2009, Cavenago et al. 2012) compared to those filled with methacrylate resin-based sealers. This phenomena may reduce the leakage of the filling. Water expansion of this material may also be one explanation to the decrease of leakage in time with epoxy resin –based sealers (Bouillaguet et al. 2008, Vasconcelos et al. 2011). Epoxy resin-based sealers are assumed to form covalent bonds between their epoxide rings and the exposed amino groups in the collagen network (Fisher et al. 2007). This bond is, however, dependent on the removal of the smear layer and exposure of dentinal collagen network.

Methacrylate resin-based sealers

Methacrylate resin-based sealers were hoped to create better adhesion to the root canal obturation core material and to root dentin. The target was to formulate the cone-sealer-dentin interface to a so-called monoblock that would efficiently fill the whole root canal apparatus. Till these days there have been four generations of methacrylate resin-based sealers. The first generation of methacrylate resin-based sealers are not in use anymore because the biocompatibility and mechanical properties proved to be poor (Kim et al. 2010).

A good example of the second generation is EndoREZ (Ultradent Products Inc, South Jordan, UT), which is a urethanedimethacrylate-based, dual-cured, non-etching hydrophilic resin sealer. It is recommended to be used with its own resin-coated gutta-percha EndoREZ points. EndoREZ has a good ability to penetrate root canal system irregularities and even deep into the dentinal tubules (Chadha et al. 2012) but high polymerization shrinkage (Bergmans et al. 2005, Hammad et al.2008, Kim et al. 2010). Polymerization shrinkage, especially with sparsely filled low-viscosity sealers, can disrupt the contact between the sealer and root canal wall (Hammad et al. 2009, Kim et al. 2010). The resulting gaps may have a dramatic effect on the microleakage of the obturation. Adhesive failure between the sealer and dentin has also been demonstrated together with low push-out bond strength when EndoRez is used (Babb et al. 2009).

The third generation for resin-based sealers included self-etching primers and a dimethacrylate-containing polycaprolactone-based filling material. Many studies available are done with Epiphany (Pentron clinical Technology, Wallingford, CT, USA), RealSeal (SybronEndo, Orange, CA, USA) and cone material Resilon (Resilon Research LLC, Madison, CT, USA). The aim was to improve the adhesion between the cone and cement. However polycaprolactone of the cone does not react with the dimethacrylate in sealer as wished because of the lack of free radicals on the cone material surface. This phenomena decreases the adhesion to the root filling material (Li et al. 2014).

In the fourth generation methacrylate resin-based sealer the etchant, primer and sealer are all put in "one bottle". RealSeal SE (SybronEndo) is the best known example of this group. It is a dual-cure, hydrophilic sealer that has a polymerizable methacrylate carboxylic acid anhydride as the acidic resin monomer. This acidic component might not be as effective to remove the smear layer compared to those that have separate acidic primer (Kim et al 2009). The push-out strength of a self-adhesive sealer is, however, higher than the strength of the second generation non-etching EndoREZ (Babb et al. 2009). The methacrylate resin-based sealers adhere to root canal dentin micromechanically by forming a hybrid layer with the collagen matrix. The use of a calcium chelating root canal irrigant improves the adhesion of methacrylate resin-based sealers to root canal dentin (Babb et al. 2009b, Kim et al. 2010) by removing the smear layer. As mentioned above the polymerization shrinkage is higher with methacrylate resin-based sealers. The resin tags may be ripped away from the tubules by the shrinkage and gaps to the dentin-sealer interface are formed (Bergmans et al. 2005) which may weaken the adhesion.

Despite extensive research to find the best materials and techniques for a perfect root filling, there seems not to be one material or technique over the other. All materials and techniques struggle with the adhesion between the two or three interfaces: dentin-sealer, sealer-root filling material and possible also sealer-cone coating (Li et al. 2014).

Calcium silicate-based sealers

A new group of sealers came to the market 2007. These calcium silicate-based sealers were hoped to have better antibacterial properties and bioactive effect on periapical healing. Their content varies widely and because of that their characteristics also vary a lot (Donnermeyer et al. 2018). The studies show wide variation for example in setting time, sealing ability, bond strength and antimicrobial efficacy (Donnermeyer et al. 2018, Silva Almeida et al 2017). Although some calcium silicate-based sealers have promising characteristics, the information of the long-term antimicrobial abilities and long-term sealing abilities is scarce (Donnermeyer et al 2018, Silva Almeida et al. 2017). The characteristics of calcium silicates are discussed in detail below.

2.1.2 Calcium silicates

Calcium silicates are osteogenic biocompatible materials (Roberts et al. 2008) commonly and widely used in dentistry nowadays. They are a group of materials with many applications, for example pulp capping, pulpotomy, apexification, repair of perforations in furcation or in root canal, repair of resorption, root filling and root end filling (Watson et al. 2014, Nilsson et al. 2013, Nayak et al. 2014, Darvell et al. 2011, Roberts et al. 2008, Martin et al. 2007, De-Deus et al. 2007, Torabinejad et al. 1995, Torabinejad et al. 1993).

In the beginning of 1990s, the first calcium silicate-based dental material, mineral trioxide aggregate (MTA), came to the market. It was approved for endodontic use in 1998. The first published case report found using Portland cement in dentistry is from 1878 (Camilleri 2015). The good properties of Portland cement were noticed, and refined Portland cement is the main component of all MTA materials. Portland cement consists mainly limestone and shale, which are burned in an oven to form tricalcium silicate, dicalcium silicate and tricalcium aluminate. Very small amounts, under 0,5%, of different metallic oxides is also found in Portland cement (Camilleri 2015). In addition MTA materials contain SiO_2, MgO, CaO, K_2SO_4 and Na_2SO_4,Fe_3O_4 (Roberts et al. 2008). Magnesium and iron are responsible of the color of MTA and possibly also the discoloration. Bismuth oxide is added to MTA for radio-opacity and it may be one factor also causing discoloration in gingival margin. Gray ProRootMTA (Dentsply Maillefer, Ballaigues, Switzerland) was the first mineral trioxide aggregate available until year 2002 when white MTA as ProRoot MTA came on the market. This white MTA contain less Al_2O_3, MgO and FeO. A smaller amount of aluminium is desirable because aluminium have been connected for example to Alzheimer's disease (Camilleri 2015).

As Portland cement contain trace amounts of some heavy metals and other undesirable chemical compounds such as lead, arsenic and chromium, the development of materials based on pure tricalcium silicate was initiated. For example Biodentine (Septodont, Saint-Maur-des-Fossés Cedex, France) and BioAggregate (Innovative BioCeramics Inc, Burnaby, BC, Canada) are products that have tricalcium silicate as the main cementous compound. The radio-opacity of Biodentine comes from zirconium oxide also used in the new tricalcium silicate-based sealers (Donnermeyer et al. 2018).

Setting of calcium silicates

All calcium silicates are hydrophilic materials and need water or humidity for the setting process, which is based on hydration of tricalcium silicate and dicalcium silicate (Camilleri 2015, Roberts et al. 2008). The setting time varies by the content of the material and the humidity of the surrounding environment (Roberts et al. 2008). For example the setting of ProRoot MTA takes from 2 to 4 hours, because the hydration reaction of tricalcium silicate, dicalcium silicate and tricalcium alumineate is quite slow and requires moisture (Darvell et al. 2011). MTA Angelus (Angelus Dental Products Industry S/A, Lindóia, Londrina, PR-Brazil) differs from ProRoot MTA with the quantity of di- and tricalcium silicates, having less dicalcium silicate and slightly more tricalcium silicate than ProRoot MTA (Camilleri 2015) and has shorter setting time, less than 50 minutes (Massi et al. 2011). According to the manufacturer (http://www.septodontusa.com) the setting time of Biodentine is 12 minutes and it does not require wet condition during setting. The setting reaction itself is supposed to be similar to the pure tricalcium silicate hydration as with MTA, except for the absence of alumina and gypsum hydration products (Watson et al. 2014).

Blood, saliva and other fluids, such as root canal irrigants, may affect the setting of calcium silicates and influence also other physical properties of these materials. Several studies have shown the negative influence of blood, blood serum and saliva on the setting time and/or the compressive strength during setting on both MTA and Biodentine (Sheykhrezae et al. 2017, Akcay et al. 2016, Kim et al. 2012, Vanderweele et al. 2006, Kogan et al. 2006). Both NaOCl and CHX decrease setting time, compressive strength (Kogan et al. 2006) and bond strength of MTA (Yan et al. 2006). The purpose of use and the influence of different circumstances and other materials on calcium silicates still need further research.

Antibacterial properties of calcium silicates

Calcium silicates, both MTA and Biodentine, have some antibacterial properties by causing highly alkalic environment, although the data of the antibacterial efficiency are divergent. Previous studies have shown that these calcium silicates had antibacterial activity against Streptococcus mutans, Escherichia faecalis, Escherichia coli and Candida, the effect being strongest against Streptococcus mutans (Bhavana et al. 2015, Koruyucu et al. 2015). According to Koruyucu et al. Biodentine and MTA Angelus had similar antibacterial activity against Enterococcus Faecalis (Koruyucu et al. 2015), while Bhavana et al. stated that the antimicrobial activity of Biodentine was superior to

MTA and glass ionomer cement (Bhavana et al. 2015). Yet another study showed MTA Angelus to have significantly higher inhibition of Str. mutans than Biodentine (Poggio et al. 2015). How long the antibacterial activity last after setting is questionable. Morgental et al. showed in their study that MTA Fillapex is effective against Enterococcus faecalis before setting, but the antibacterial effect did not exist after 7 days (Morgental et al. 2011). Prestegaard et al. showed also a decreasing tendency of antibacterial effect in seven days also with ProRoot MTA (Prestegaard et al. 2014).

Bioactivity of calcium silicates

Bioactivity of a biomaterial is important for the healing process. Bioactive materials can leach ions, that have an important role in biomineralization (Vallittu et al. 2018a). Calsium silicates are a good example of bioactive materials. These bioactive materials have the ability to form carbonated apatite on their surface in the presence of body fluids (Vallittu et al. 2018a). MTA and especially the Portland cement compound immersed in phosphate-buffered saline can form an initial amorphous calcium phosphate phase that hydrolyses to an apatite phase. The high pH, which was over 9,25, had a positive effect on this formation (Tay et al. 2007). Kim et al. show in their study the bioactivity of both Pro Root MTA and Biodentine in simulated body fluid, where the phosphate concentration is the same as in blood plasma. With both materials there has been shown to be a separate interfacial layer between the cement and dentin. Amorphous calcium phosphate is formed of the surface of the material and this is the precursor of the formation of carbonated apatite, indicating biomineralization at the interface (Reyes-Carmona et al. 2009, Kim et al. 2015, Han and Okiji 2011).

2.2 Root canal posts and post cementation

2.2.1 Root canal post

In restorative dentistry only the crown restauration is not always enough. We need endodontic treatment and root canal posts are needed to support the restauration. The post must bear masticatory forces from different directions and its elastic modulus should be near the one in dentin (about 20 GPa) (Poolthong et al. 2001). Extensive research has been conducted to find the most durable combination of post and core/restoration not forgetting the esthetic point of view. Almost till the beginning 19^{th} century the prefabricated metallic posts and metallic cast posts were the only posts on market. After that numerous different kind of fiber posts have been brought into the market. The first fiber post was based on carbon fibers attached to the matrix with epoxy resin (Duret and Duret 1990). They were passive in shape and designed to be used with bonding technique (Fredriksson et al. 1998). The survival rate of these posts was quite high (over 70%) (Ferrari et al. 2000b, Glazer 2000, King et al. 2003). The failures were mostly not related to the post itself and were more often reparable than with metal posts (Glazer 2000, Ferrari et al. 2000b). In these studies the failure with carbon fiber posts were usually endodontic failure or debonding of the crown or the post. The characteristic strength of carbon fiber posts is similar to the cast posts but the modulus of elasticity is lower (Pereira et al. 2014).

From the esthetic point of view, the carbon fiber post had its limitation with the dark color. Ceramic posts have been on the market from the late 19th century. Zirconium posts are very strong (the strength being similar to the titanic post), but the elasticity is low, which indicate that these posts are brittle and have no ductility. Because of the hardness of zirconia they can be difficult to remove if re-entry is needed (Asmussen et al. 1999, Schwartz and Robbins 2004). There has also been problems with retention to composite cores with zirconia (Butz et al. 2001).

The demand of posts with more plasticity increased because the rigidity of the posts may cause severe root fracture (McLaren et al. 2009, Fokkinga et al. 2004, Schwartz and Robbins 2004). In addition, the minimaly invasive preparation was not always easy to achieve with metallic or ceramic posts. The use of fiber reinforced composite posts (FRC post) has largely replaced the metallic and ceramic posts in restorative dentistry nowadays although the Cochrane analysis failed to prefer one technique to the other (Bolla et al. 2007). Also a resent review by Marchionatti et al. found no difference in survival rates between fiber posts and metal posts (Marchionatti et al. 2017). In FRC posts the strengthening fibers are bound together with polymer matrix. The mechanical properties of these posts depend on the type of fibers and the compound of the matrix. Prefabricated FRC posts are often used with the same technique as metallic posts, where the post and restoration have to bear mostly vertical forces (Vallittu 2016). Several studies have shown that the major failure type of FRC posts is debonding from the dentin or the cement but the failures are often reparable (Marchionatti et al. 2017, Fokkinga et al. 2004, Naumann et al. 2005, Salameh et al. 2007, Le Bell-Rönnlöf et al. 2011). The fracture strength and stress distribution of teeth restored with fiber posts are also higher (Hayashi et al. 2006, Hayashi et al. 2008) and less sensitive to post diameter and length (Rodriguez-Cervantes et al. 2007) than with metal or ceramic posts. On the other hand debonding of the post may cause severe problems as secondary caries and root canal reinfection may occur (Lancaster 2015, Barfeie et al. 2015, Bolla et al. 2007). It has been proposed that the flat surface of dentine is not durable enough unless the post system is able to carry the load and diminish the tensile stress at the crown margin (Vallittu 2016).

A way to reduce the problems related to debonding of prefabricated FRC posts could be the use of individually formed FRC posts. In these posts, the fibers are typically impregnated with a semi-interpenetrating polymer network (semi-IPN) which means that it has a polymer network containing both cross-linked and linear polymer structures that makes it possible to form interdiffusion bonding with the resin in cement (Le Bell et al. 2004). The individual semi-IPN post show higher bonding between the post and cement (Le Bell et al. 2005) and higher flexural properties (Lassila et al. 2004) than the prefabricated posts (Vallittu 2018b).

2.2.2 Luting agents used in fiber post cementation

Luting agents used in fiber post cementation play a remarkable role in the preservation of the post adhesion (Ubaldini et al 2018). Failure in the post adhesion can cause failure of the whole restoration, increase the possibility of microleakage and jeopardize the endodontic treatment. The present resin cements can be divided by the curing mechanism or by the adhesive system used. There are three different curing mechanisms among the resin cements: 1) light-cured, 2) chemically / self-cured or 3) dual-cured, which is a combination of both earlier mentioned. Only light-cured resin cements are not used for post cementation because of the limited light access to the deep root canal (De Souza et al. 2015). Depending on the type of cement, they contain different activators (photo or chemical initiator or both) to initiate the polymerization. These activators/initiators break the molecules carbon-carbon double bonds to release free radicals that initiate the polymerization (Calixto et al. 2012). The converted monomers build a polymeric chain. The maximum amount of double bonds converted to single bonds is called the degree of conversion and is often used to describe the degree of polymerization (De Souza et al. 2015). Dual-cured resin cements need both the photoinitiator and chemical initiator and the light curing is always needed for proper polymerization. If light curing is not used, the degree of conversion may be low and the mechanical properties of the cement decrease (Pick et al. 2010, Arrais et al. 2007). It is also notworthy that post-polymerization increases the dual-cure cement bond strength for several hours after initial polymerization. (Faria-e-Silva et al. 2010).

The other way of looking at the luting agents is the adhesive mechanism used with the cement. The cement may be used with etch-and-rinse adhesive system, self-etch adhesive system or it can itself be self-adhesive (Gopal et al. 2017, Farina et al. 2016, Sarkis-Onofre et al. 2014). Etch-and-rinse adhesive bonding is analogous to coronal resin restoration bonding: the canal is first etched with

37% phosphoric acid, the excess amount of water is dried leaving the root dentin moist, and the adhesive is applied by the instructions of manufacturer. The problems with this system are for instance the moisture control in root canal, the access of the adhesive to the apical part of the root (Ferrari and Mannocci 2000a, Perdigao et al. 2007) and the lower degree of conversion, which as such may also be a positive character because it usually means lower polymerization shrinkage (De Souza et al. 2015).

The luting cement with self-etching adhesive (often called self-etch cement) require a self-etch primer to be applied before the cement. In this technique the self-etching primer is usually applied on dry dentin and do not require rinsing with water. Self-etch cements have significantly thinner hybrid layer compared to that with separate etching with 35% phosphoric acid (Bitter et al. 2004). One could speculate that this may affect the bonding to root dentin by leaving thicker smear layer on the root canal walls. On the other hand Tay et al. 2000 have shown that the thickness of smear layer did not influence the adhesive capacity of self-etch adhesive system (Tay et al. 2000).

The self-adhesive cements do not need any pretreatment. They contain functional monomers such as MDP-10 (methacryloxy-decyl-dihydrogen-phosphate) and 4-META (4-methacryloxyethyl trimellitate anhydride). These acidic monomers bind to calcium in the hydroxyapatite of the demineralized dentin creating a bond to the resin network (Al-Assaf et al. 2007). The acidic monomers in these self-adhesive cements may decrease the degree of conversion of the self-adhesive dual-cured cements because they seem to interact with the amine initator in dual-cured resins. This phenomenon can negatively affect the mechanical properties of these cements (De Souza et al. 2015, Vrochari et al. 2009). On the other hand, this could also be an explanation for the low polymerization shrinkage of these cements (De Souza et al. 2015). Many studies show good bonding properties with self-adhesive cements (Pereira et al. 2014, Frassetto et al. 2012, Bitter et al. 2006) although there are studies that show the opposite (Temel et al. 2017, Calixto et al. 2012, De Munck et al. 2004). In any case, it could be stated that the usage of self-adhesive cements minimizes the possible technical errors because of their ease to use (Skupiei et al. 2015).

2.3 Mechanisms and factors affecting adhesion in root canal

2.3.1 Material dependent challenges of immediate bonding to root canal dentin

Various factors may complicate the bonding to root canal dentin. The anatomy of the root canal system makes it more difficult to get the adhesives to the most apical parts of the root. Also the moisture control on every part of the root and a uniform layer of the adhesive material on every part of the root canal may be challenging to maintain. The data of the bond strength in different parts of root canal vary: some studies show lower bond strength in the apical parts of the roots (Rodrigues et al. 2017, Calixto et al. 2012), similar bond strength in every part of root canal (Kul et al. 2016) or higher bond strength in apical part of the root (Bitter et al. 2006). Exclusively light-cured materials are not suitable to use in root canal because it is impossible to reach every part of the root canal with the light. Even dual-cured materials need to be properly photo-activated to achieve the highest possible degree of conversion (De Souza et al. 2015). Thickness of the smear layer in the root canal may also influence the adhesion to root canal dentin, but there is no general agreement of this. There are studies for (Wu et al. 2009) and against (Carvalho et al. 2017, El-Ma'aita et al. 2013, Tay et al. 2000) the influence of smear layer on the bond strength. The effect of the material penetration to the dentinal tubules on the adhesion to root dentin is similarily a disputed issue (Ubaldini et al. 2018, Violich and Chandler 2010).

A great deal of research has concentrated on the influence of the root canal irrigants on the adhesion to root canal dentin. Commonly used irrigants such as sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX) are the most researched endodontic irrigants. NaOCl may create an oxygen layer on the root canal dentin and by that it is possible that it can decrease the bond strength. Several studies have shown that 5,25 % NaOCl may disturb the bonding (Weston et al. 2007, Santos et al. 2006, Erdemir et al. 2004) but the effect may be concentration-dependent since other studies with NaOCl have failed to show the difference (Vilanova et al. 2012, Cecchin et al. 2010, Muniz and Mathias 2005). The higher concentrations of NaOCl (5,25% or more) may decrease the bond strength at least with post cementation when self-adhesive cements are used (Bueno et al. 2016, Varela et al. 2003). Bueno et al. and Varela et al. speculated that this is possibly due to the oxidation process or the self-adhesive cement is not strong enough to demineralize the smear layer on root canal walls. EDTA is used to remove the smear layer and as mentioned above there is no consensus of its effect on bond strength in root canal. CHX have not been shown to negatively affect the bond strength of coronal fillings or post bonding (Santos et al. 2006).

The bonding mechanism of resin cements, root canal sealers and tricalcium silicates, to the root dentin is different which makes the bonding even more challenging.

Bonding mechanism of resin luting cements:

Resin luting cements can be divided into three categories: 1) 3-step etch-and-rinse adhesive combined with dual cured resin cement, 2) 1-step self-etch adhesive combined with dual cured resin cement or 3) self-adhesive dual cured cement. In the adhesion of group 1, the acids demineralizes the dentin so that the hydrophilic monomers can infiltrate the dentin and form the hybrid layer (Rodrigues et al. 2017, Sarkis-Onofre et al. 2014). In group 2 1-step self-etch adhesives contain a functional monomer that can promote a chemical interaction with hydroxyapatite and also forms a micromechanical hybridization (Rodrigues et al. 2017, Shibata et al. 2016, Sarkis-Onofre et al. 2014). Self-adhesive cements bond to dentin without any separate adhesive system. They demineralize the dentin without formation of the hybrid layer or resin tags. The acidic monomers of these cements bind with calcium in the hydroxyapatite to form a stabilising attachment between the methacrylate network and dentin. Besides this chemical bonding the polymerization also leads to micromechanical retention (Rodrigues et al. 2017, Sarkis-Onofre et al 2014, Ferracane et al. 2011).

Bonding mechanism of root canal sealers

The adhesion of root canal sealers to root dentin vary by the character of the sealer. Epoxy resin-based materials are assumed to bond with covalent bonds between their epoxide rings and the exposed amino groups in the collagen network (Fisher et al. 2007), so they are at least partly forming a chemical bond to the dentin. Methacrylate-based sealer materials act more like dental adhesives. These materials depend on micro-mechanical inter-locking with the collagen matrix for retention (Fisher et al. 2007).

Bonding mechanism of tricalcium silicate cements

Calcium silicate cements release calcium hydroxide during setting reaction. In the presence of phosphate from tissue fluids, a layer that resembles hydroxyapatite is formed between the root dentin and calcium silicates. These cements also form tag-like structures beside the interfacial layer, which is called the “mineral infiltration zone” (El-Ma'aita et al. 2013, Reyes-Carmona et al. 2010). In this zone the caustic effect of the alkaline of calcium silicates degradate the collagenous component of the interfacial dentin. This degradation leads to a porous structure which facilitates the permeation of Ca^{2+}, OH^-, and CO_3^{2-} ions leading to increased mineralization in this region. So the adhesion between calcium silicates and root dentin is of chemical character (Atmeh et al. 2012).

2.3.2 Bonding durability

All adhesive systems function by hybridization process. This means the micromechanical interlocking of the adhesive to dentin and the formation of hybrid layer, a structure where the resin matrix is infiltrated between the demineralized collagen fibrils (Breschi et al. 2018). Water is an important part of this process preventing the etched collagen network from collapsing. The hydrophilic monomers replace the water and infiltrate the collagen layer to form the hybrid layer (Sartori et al. 2015, Tjaderhane et al. 2013c). In long term, water can also harm the hybrid layer by causing hydrolysis (Breschi et al. 2018, Sartori et al. 2015).

Hydrolysis is a chemical reaction where the chemical compounds resolve by the addition of water. In adhesive dentistry two types of hydrolysis are recognized: 1) Hydrolysis of resin based polymeric matrix (Anchieta et al. 2015). 2) Enzymatic hydrolysis or enzymatic degradation, where exposed collagen fibrils are not covered by the adhesive and are available for enzymatic degradation (Tjaderhane et al. 2013c). In dentin bonding the important aim is to achieve a durable hybrid layer of the dentin collagen fibrils and the adhesive. The adhesive ought to fill all interfibrillar spaces of demineralized dentin. It is however most unlikely that all water molecules between collagen fibrils could be replaced by polymeric resins although they are water miscible. There are small nanovoids which can cause water leakage. This nanoleakage may promote the hydrolysis of the resins and the collagen matrix and within time it may cause the displacement of the filling material. (Tjaderhane et al. 2013c)

Hydrolysis of resin based polymeric matrix

Contemporary composite filling materials, materials used in root canal obturation and cements used in root canal contain different amount of hydrophilic monomers. They are necessary for dentin wet bonding to achieve proper hybridization of collagen matrix. Most common hydrophilic methacrylate used in adhesives is hydroxyethyl methacrylate (HEMA) (Van Landuyt et al 2007). HEMA improves the bonding by wetting the dentin, but it is also vulnerable to water degradation (hydrolysis) (Van Landuyt et al 2007). Especially one-step self-etch adhesives contain water and hydrophilic monomers to improve the immediate resin-dentin bonding. These hydrophilic adhesives absorb water, which causes swelling of the polymer and weakening of the polymer network and significant correlation between water sorption and decrease in elastic modulus and tensile strength has been demonstrated (Hosaka et al. 2010, Anchieta et al. 2015). The higher the HEMA concentration the more water sorption and hydrolytic degradation of the adhesive occurs (Hosaka et al. 2007).

Some salivary enzymes can also cause hydrolysis of other polymers. For example dental resin monomers, BisGMA and TEGDMA may be degraded by the salivary esterases at their ester linkage. In human saliva, proteins like albumin, has been shown to enhance esterase activity. This kind of biodegradation may decrease the mechanical properties of the composite filling. (Cai et al. 2014)

Enzymatic hydrolysis

Degradation of the collagen, the other component in hybrid layer, has been under active research in recent years. A lot of work has been done to identify the enzymes involved in this action. Two groups of endogenous enzymes, matrix metalloproteinases and cysteine cathepsins, have been shown to be the most important. (Tjaderhane et al. 2013c, Tjaderhane et al. 2013b, Breschi et al. 2018). The enzymatic hydrolysis is discussed below.

2.4 Enzymatic activity

2.4.1 Matrix metalloproteinase enzymes, MMPs

MMPs are a family of zinc- and calcium–dependent endopeptidases that can degrade extracellular matrix components. They contribute in both normal and pathological conditions by remodeling the tissue. MMPs are capable of breaking down the collagen fibers and by that they take part of many pathological conditions with connection to collagen for example caries, pulpal and periapical infections, periodontal diseases and oral tumors (Sorsa et al. 2004). In addition, the collagenolytic activity can weaken the adhesion between adhesive materials and dentin by degrading the collagen of the hybrid layer (Tjaderhane et al. 2013c). MMP enzymes are also connected to bone resorption by degrading the collagen remnants that have been exposed by the action of osteoclasts (Hannas et al. 2007).Twenty three different MMPs are found in humans. They are classified by their function or structure. MMP-1, MMP-8 and MMP-13 that break down the collagen fibrils are called collagenases. Gelatinases MMP-2 and MMP-9 degrade gelatin, which is a denatured form of collagen. MMP-3 and MMP-10 are called stromeolysins and MMP-3 is critical for the activation of proMMP-1, an inactive form of MMP-1. Matrilysins MMP-7 and MMP-26 lack the hemopexin domain (Figure 1). Membrane-type MMPs are a group of six MMPs that digest extracellular matrix and some of them activate proMMP-2. In addition of these there is a group called other MMPs which are enzymes that are not classified in the previous mentioned groups. (Visse and Nagase 2003)

Human odontoblasts express a wide variety of MMPs (Palosaari et al. 2003). Human dentin has been shown to contain at least both gelatinases MMP-2 (de Las Heras et al. 2000) and MMP-9 (Mazzoni et al. 2007), collagenase MMP-8 (Sulkala et al. 2007), stromeolysin MMP-3 (Mazzoni et al. 2011, Boukpessi et al, 2008) and matrilysin MMP-7 (Mazzoni et al. 2018). MMP-20 is also found in dentin, but it may have a bigger role in dentin caries progression and dentin formation (Sulkala et al. 2002). MMP-8 is the major collagenase entzyme in human dentin (Sulkala et al. 2007). MMPs are found not only in coronal dentin but also in radicular dentin (Santos et al. 2009, Toledano et al. 2010, Tay et al 2006).

The structure of all MMPs is quite the same. They all have a prodomain part which is linked to a catalytic domain including a zinc (Zn^{2+}) binding site (Figure 1). All of the MMPs present in human tooth contain also a hemopexin domain which mediates with substrate specifity and protein-protein interaction (Tjaderhane et al. 2013c). MMPs -2 and -9 contain also a fibronectin-like domain with special affinity to gelatin. The prodomain keeps the enzyme as an inactive zymogen until this part is removed by proteolysis. This activation of MMPs can be achieved by autocatalysis, proteolytic enzymes and some chemical agents or by changes of pH (Hannas et al. 2007, Nishitani et al. 2006).

The influence of adhesives on MMPs in dentin

In mineralized dentin, MMPs are covered with apatite crystals that keeps them in dormant state. Previously it has been shown that in very low pH MMPs may be inactive, but even in adhesive dentistry commonly used 37% phosphoric acid does not permanently denature them (Tezvergil-Mutluay et al. 2013). It has also been shown that etch-and-rinse-adhesives (Mazzoni et al. 2006) and weak self-etching adhesives, with pH between 2.3 to 4.6 can activate MMPs without denaturing the enzymes (Nishitani et al. 2006, Tay et al. 2006). This phenomen may adversely affect the longevity of composite restorations (Carrilho et al. 2007a, Carrilho et al. 2007b), root canal fillings and post cementation (Tay et al. 2006).

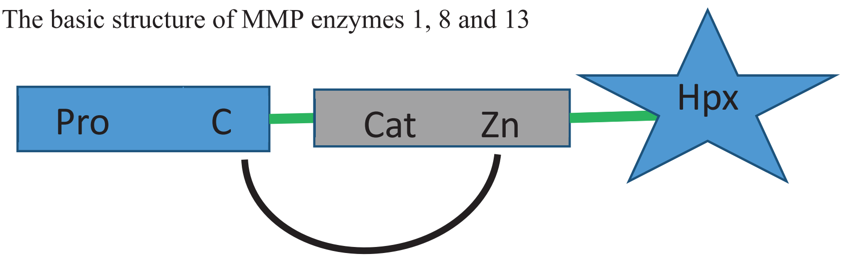
As the MMPs are hydrolases, they need water to hydrolyze the peptide bonds in collagen. It has been thought that weakly acidic all-in-one adhesives, that contain hydrophilic resin monomers to properly infiltrate the collagen, absorb water, activate MMPs and undergo hydrolytic degradation with time. This could probably at least partially be prevented by using self-etching primer and covering the exposed collagen with a hydrophobic adhesive to lower the water penetration into the hybrid layer. (Nishitani et al. 2006)

MMPs can also be regulated by specific tissue inhibitors of metalloproteinases (TIMPs) (Brew et al. 2000). TIMPs are small proteins that can attach to the active site of MMP (usually the hemopexin) and possibly inhibit the activation of the latent form of it (Visse and Nagase 2003, Hannas et al. 2007). Inactivation of these TIMPs occur also in time and so-called autoactivation of MMPs starts. Acid-etch and acidic monomers in dentin-bonding agents could possibly activate the MMPs by removing the inhibiting TIMPs from these MMPs (Mazzoni et al. 2013).

Other studies have shown that even the 37% phosphoric acid may not denature the endogenous proteases of the dentin. It has been speculated that the decrease of MMP activity, when using 37% acid, may partly be by creation of $CaHPO_4$ precipitates over the enzymes. (Tezvergil-Mutluay et al. 2013). These findings only show how complicated the adhesion to dentin is.

Figure 1.

The basic structure of MMP enzymes 1, 8 and 13



Pro: Prodomain containing cysteine

C: Cysteine, which is linked to the zinc atom and holds the enzyme in a latent form until this link is broken.

Cat: Catalytic domain with zinc atom

Zn: Zinc atom

Hpx: Hemopexin

2.4.2 Cathepsins

It was over 50 years ago, when lysosomes were discovered. These small particles were rich in hydrolytical enzymes, hydrolases. About 50 different lysosomal hydrolases has been found. Among these are the cysteine cathepsins, which are members of papain-like cysteine proteases. Cysteine cathepsins have a big role among the lysosomal hydrolases because they are widely expressed in human tissues and they regulate many important biological processes. Eleven human cysteine cathepsins has been detected (B,C,F,H,K,L,O,S,V,X,W). Cathepsins B, H, L, C, X, F, O and V are involved in normal cellular protein degradation and turnover. Cathepsins K, W and S are more tissue-specific and have more specific roles. Cathepsin K is the most potent mammalian collagenase and is highly expressed in osteoclasts. It plays an important role in bone resorption and remodeling both in normal and pathological conditions. Cathepsin B can degrade extracellular matrix components such as type IV collagen and fibronectin. Cathepsins require a slightly acidic environment to be optimally activated. In neutral pH most of the cathepsins are unstable. Cathepsin S makes an exception to this by being stable at neutral or slightly alkaline pH. The interaction of cathepsins and MMPs is very interesting and under active research, but the potential interactions between these two extracellular matrix degrading proteinases are still unknown. It is known that cathepsins are capable of increasing the activity of MMP enzymes by inhibiting the function of TIMPs, but they can also work the other way down so that MMPs activate the cathepsin (for example procathepsin B) (Turk et al. 2012, Tjaderhane et al. 2013c).

Tersariol et al. showed in 2010 the presence of cathepsins in sound dentin and dentinal tubules. They found a wide expression of cysteine cathepsins both in odontoblasts and in pulp tissue (Tersariol et al. 2010). Cathepsin B has also been connected not only to cancer but also to gingivitis and periodontitis (Kennett et al. 1997). Nascimento et al. found increased activity of cathepsin B in carious dentin compared to intact dentin and the activity was higher with active and deep lesions with younger patients. They also found highly significant correlation between MMPs and cysteine cathepsins, which indicates that they both have an important role in dentinal caries pathogenesis. Cysteine cathepsins might also activate the MMPs establishing the synergy of these two enzymes (Nascimento et al. 2011). 2014 Vidal et al. have shown that cathepsin K is present in both intact and carious dentin. They have also shown the multiple abundance of cathepsins B and K and MMP-2 and -9 in caries affected dentin compared to intact dentin. Together with the co-distribution of MMPs and cathepsins in intact dentin this result support the notice that cysteine cathepsins and MMPs may work together in pathophysiological processes where the dentin organic matrix remodeling takes place (Scaffa et al. 2017, Vidal et al. 2014). During caries process, erosion and forming hybrid layer between composite and dentin, the environment becomes lightly acidic due to the bacterial function or the acids used in resin-dentin bonding (Tjäderhane et al. 1998, Tjaderhane et al. 2015, Scaffa et al. 2017). Cysteine cathepsins are autoactivated in low pH and can activate the latent proMMPs (Turk et al. 2012), which become functional when the surrounding pH is neutralized due to salivary buffers (phosphate and calcium) or dentinal fluid, resulting with the degradation of collagen, which in restorative dentistry means the degradation of the hybrid layer (Tjaderhane et al. 2015, Scaffa et al. 2017).

2.4.3 Collagenolytic Enzyme inhibition and adhesive dentistry

In adhesive dentistry continuous efforts has been made to find a way to maintain the collagen matrix integrity in the hybrid layer between the restorative materials and dentin. The main research has been directed on the inhibition of endogenous dentin proteases, matrix metallo proteinases MMPs and cysteine cathepsins, to improve the denting-bonding durability and to create long-lasting restorations. There are two kind of inhibitors that can reduce the activity of dentin proteases: endogenous and exogenous. As earlier mentioned dentin contains TIMPs, which are endogenous inhibitors that regulate the action of the enzyme (Visse and Nagase 2003). Cystatins are the main endogenous cysteine cathepsin inhibitors (Turk et al. 2012), but their presence in human dentin is not known.

Most of the exogenous inhibitors chelate calcium or replace zinc ions at the MMP propeptide and others may prevent MMP access by coating the substrate (Mazzoni et al. 2012, Tjaderhane et al. 2013b). Tetracycline and doxycycline have been shown to inhibit MMPs. Bisphosphonates, e.g. Galardin inhibit MMPs by chelating active-site zinc. They are used to treat conditions involving increased bone resorption like osteoporosis (Mazzoni et al. 2015, Tjaderhane et al. 2013b). Some antibicrobial compounds incorporated to adhesives can also inhibit MMPs in hybrid layer. Polymerizable quaternary ammonium methacrylates have shown to have inhibitory effect and may have positive effect on bond strength (Mazzoni et al. 2015, Tjaderhane et al. 2013b). Benzalkonium chloride, known as BAC, 0.5% have been shown to completely inhibit MMP-2, -8 and -9 without affecting the immediate bond strength (Tezvergil-Mutluay et al. 2011). Functional monomer like 10-MDP (10-metacryloyloxydecyl dihydrogen phosphate) create a chemical bond to calcium ions of the hydroxyapatite crystals. It was thought that mild self-etch adhesive with 10-MDP would minimize the nanoleakage and leave a thin layer of hydroxyapatite on the collagen fibrils to mask the collagen cleavage site and keep the enzymes fossilized (Tjaderhane et al. 2013b). Cross-linking reagents, molecules that contain one or more reactive terminations capable of chemically attaching to specific functional groups on proteins (Mazzoni et al. 2015), are hoped to strengthen the collagen network, inactivate the MMPs and reduce the collagen degradation (Mazzoni et al. 2015) (Tjaderhane et al. 2013b).

EDTA

Ethylenediaminetetraacetic acid (EDTA) is a calcium chelator that is widely used in endodontic treatment to remove smear layer from the root canal walls. 17% EDTA can significantly decrease the MMP activity in dentin after 1 minute exposure time (Thompson et al. 2012). However, EDTA is so water soluble that it can very easily be rinsed off the dentin which makes EDTA not as efficient as MMP inhibitor as some other substances (Carrilho et al. 2009).

This research has concentrated on two irrigants that act as MMP inhibitors: chlorhexidine, which is widely used for different purposes in dentistry and dimethyl sulfoxide, a solvent that has recently gained interest in adhesive dentistry and that might have potential use in dentistry in future.

Chlorhexidine

CHX is widely used in endodontics because of its antimicrobial properties. Its inhibitory effect on MMPs has been shown in numerous studies not only in composite adhesion in coronal dentin cavities but also in radicular dentistry. In 1999 Gendron et al. showed that MMP-2 and -9 could be inhibited with low concentration of CHX, possibly via calcium chelating mechanism at least with lower concentration (Gendron et al. 1999). With higher concentration of CHX the mechanism may be protein denaturation. In addition to MMP inhibition, CHX is also a very potent inhibitor of the cysteine cathepsins present in dentin-pulp complex (Scaffa et al. 2012).

Numerous studies have shown CHX to be beneficial for long-term adhesive-dentin bond with composite fillings (Pashley et al. 2011b, Tjaderhane et al. 2013b, Tjaderhane et al. 2015, Breschi et al. 2018). With root canal posts the bond strength-preserving effect of CHX is not seen as clearly as it can be seen with composite restorations and the statements of the effect of CHX on the post bond strength/luting cement adhesion in root canal vary a lot. Some studies have shown that CHX may at least moderately improve the immediate and the long-term bond strength with post cementation (Cecchin et al. 2014, Shafiei and Memarpour 2010, Cecchin et al. 2011), while others have show no influence on the long-term post bond strength when using CHX (Angeloni et al. 2017, Leitune et al. 2010). Some studies even indicate that CHX could have negative effect on immediate or long term push-out bond strength (de Araujo et al. 2014, Hiraishi et al. 2009). In the study of Hiraishi et al the negative effect of CHX was found in the apical part of the root (Hiraishi et al. 2009), where the bond strength even without CHX may be weaker (Aksornmuang et al. 2007, Bitter et al. 2004). The root canal system is a complex system to study bond strength. In the studies mentioned above study protocols vary (push-out bond strength, microtensile bond strength), some studies measure bond strength in different parts of the root (coronal, middle, apical) while some have not specified the location in the root canal. In addition most of these studies have not taken into consideration the actual location of the debonding to discriminate the failures between the post and cement or between the cement and root dentin. Nevertheless there still is no consensus of the effect of CHX on the bond strength to root dentin with post cementation.

Dimethyl Sulfoxide

DMSO is a solvent that has been used as a penetration enhancer in industry and pharmacology from 1960s. It has an ability to deliver both hydrophilic and lipophilic medications through skin (Marren 2011). In human medication up to 50% DMSO concentrations and in veterinary medicine as high as 90% concentrations are used (Marren 2011). Recent studies suggest that DMSO could improve both immediate and long-term adhesive bond strength in dentin (Stape et al. 2016a, Stape et al. 2015, Tjäderhane et al. 2013a) partly by inhibiting the function of MMPs (Tjäderhane et al. 2013a). The increase in immediate bond strength may also relate to DMSO`s ability to reduce dentin surface free energy, improving wettability (Mehtala et al. 2017) and adhesive penetration (Stape et al. 2015). These may be related to DMSO's ability to break down water's self-association forming stable complexes with water molecules (Vishnyakov et al. 2001), which has been suggested to allow cluster interaction of adhesive monomers with hydrated dentin collagen (Stape et al. 2016b).

3. HYPOTHESIS AND AIMS OF THE STUDY

The aim of the study was to analyse the immediate and long-term effects of chlorhexidine (CHX) and/or dimethyl sulfoxide (DMSO) on the adhesion and bonding ability of fiber reinforced root canal posts, resin cement, sealer and calcium silicate cements. The hypothesis was that neither CHX nor DMSO would affect the immediate or long-term bond strength, microleakage or adhesion of the tested materials.

The specific objectives were:

1. To measure the immediate push-out bond strength of fiber reinforced root canal posts to root dentin, with the special interest to the effect of chlorhexidine on the bonding and the fracture pattern (I).

2. To evaluate the effect of chlorhexidine on the bonding and fracture pattern of FRC posts to root dentin after aging (II).

3. To evaluate and compare the immediate and long-term effect of CHX and DMSO on the microleakage of two sealers (III).

4. To evaluate and compare the immediate and long term effect of CHX and DMSO on the push-out strength and microleakage of two calcium silicate cements and to determine the changes in fracture modes caused by the tested irrigants within time (IV).

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Teeth

In all studies human third molars that were extracted in the University Student Health Care Centre in Tampere and Oulu, and the Unit of Specialised Oral Care in the City of Helsinki, Finland were used. The teeth were used for the study with the patients' consent and approval from the Ethical Committee, Faculty of Medicine, University of Oulu. Teeth were stored in 0.2% sodium azide at 4°C until used. Third molars with one separate root with minimum length of 9 mm were selected.

4.1.2 Post materials (Studies I and II)

Prefabricated fiber reinforced posts

The FRC posts used in studies I and II were glass fiber posts (unspecified glass fibers, E-glass fibers, zirconium glass fibers or quartz fibers). The formation of the fibers were either continuous unidirectional or multi-axial braided plait. The fibers were embedded in epoxy resin matrix. The posts were either light-transmitting or translucent and radio-opaque, depending on the manufacturer. The surface of the posts were microporous, pre-activated/pre-silaneted or smooth. The post diameter of all posts was 1.5 mm and if the post point was tapered, the measurements was done on the untapered part of the post. (Table 1.)

Individually formed fiber reinforced post

The individually formed post used in studies I and II was soft and flexible glass fiber post that was resin-impregnated with polymer (PMMA and bis-GMA). The fibers were silanated E-glass fibers. The silane coupling agent improves the adhesion between the matrix polymer and the glass fibers. This is based on two types of bonds: 1) a siloxane bridge can be formed by condensation reaction between silanol group and the glass surface; 2) a bond between silane coupling agent and resin matrix may be formed by copolymerization if the silane coupling agent has functional groups similar to those found in methacrylate resin. The matrix of this post is so called semi-interpenetrating polymer network polymer matrix. This means that the polymer contains one or more polymer network and also linear or cross-linked polymers which attach on the molecules of at least one network. (Vallittu,P.K. 2015, Vallittu,P.K. 2000) (Table 1.)

Table 1. The post materials/data from the manufacturers

Post Brand	Manufacturer	Fiber	Matrix	Post type	Post surface
Glassix Post	Harald Nordin SA, Chailly-Montreux, Switzerlad	Glass fiber	Epoxy resin	pre-fabricated	smooth
D.T.Light-Post	Bisco Inc, Schaumburg, IL, USA	Quarts fiber	Epoxy resin	pre-fabricated	smooth
UniCore Post	Ultradent, Salt Lake City, UT, USA	Zirconium glass fiber	Resin type?	pre-fabricated	microporous
EverStickPOST	Stick Tech Ltd, Turku, Finland	E-glass fiber	Bis-GMA PMMA	individually formed	smooth

4.1.3 Luting cements (studies I and II)

All three cements used in these studies were methacrylate based dual cured composite cements. They can be chemically cured but activated with light. RelyX Unicem was the only self-adhesive cement in this study. All composite resin luting cements, etchants and adhesives were used according to the recommendation of manufacturers presented in Table 2.

Table 2. Luting cements and bonding agents

Luting agent	**Manufacturer**	**Bonding agent**	**Composition of composite resins**	**Primer/bonding agent**	**Conditioning method**
Duo-link	Bisco, Inc., Schaumburg, IL, USA	All-bond 2: Primer A+B, Pre-Bond Resin	Bis-GMA, TEGDMA, glass filler, UDMA	Primer A: NTG-GMA, acetone, ethanol, water Primer B: BPDM, photo-initiator, actone Pre-Bond:Bis-GMA, TEGDMA, BPO, HEMA	ULTRA-ETCH (35% phosphoric acid)
Perma Flo DC	Ultradent, Salt Lake City, UT, USA	PermaFlo DC	TEGDMA, Bis-GMA	Primers A and B, Primer A: NTG-GMA, ethanol 40, acetone 50; Primer B: proprietary indirect luting/bonding primer, ethanol 20, acetone 50	ULTRA-ETCH
RelyX Unicem	3M ESPE, Seefeld, Germany	No bonding agent	Silica, glass, calcium hydroxide, methacrylated phosphoric ester, dimethacrylate acetate		Self-adhesive resin cement
Ultra-etch (35% phosphorid acid)	Ultradent, Salt Lake City, UT, USA				
Stick Resin	Stick Tech, Turku, Finland	Adhesive on EverStickPOST			

BIS-GMA – Bisphenol-A-glysidyl methacrylate

TEGDMA – Triethylene glycol dimethacrylate

NTG-GMA – N-tolylglycine glycidyl methacrylate

UDMA – Urethane dimethacrylate

HEMA – 2-hydroxyethyl methacrylate

BPDM – Biscois own patented hydrophilic monomer

BPO – Benzoyl peroxide

4.1.4 Root canal obturation materials (studies III and IV)

Three different sealer materials, master points and accessory points were studied and used according to each manufacturer's protocols (Table 3).

In study IV the whole root canal was filled with tricalcium silicate based cements ProRoot MTA and Biodentine. Both materials were used by the recommendation of manufacturers (Table 3).

Table 3. Obturation materials used in studies III and IV

Brand	Manufacturer	Type of material
RealSeal SE	SybronEndo, Glendora, Canada	methacrylate resin based self-etching sealer
RealSeal Points #35	SybronEndo, Glendora, Canada	Resilon (polyester compound, difunctional methacrylate resin, bioactive glass, radiopaque filler)
RealSeal accessory point M	SybronEndo, Glendora, Canada	Resilon (polyester compound, difunctional methacrylate resin, bioactive glass, radiopaque filler)
Topseal	Dentsply Maillefer, Ballaigues, Switzerland	epoxy resin
Gutta-percha master cone # 35	Dentsply Maillefer, Ballaigues, Switzerland	gutta-percha
Gutta-percha accessory point C	Dentsply Maillefer, Ballaigues, Switzerland	gutta-percha
ProRoot MTA	Dentsply Maillefer, Ballaigues, Switzerland	Portland cement, bismuth oxide
Biodentine	Septodont, Saint-Maur-des-Fossés Cedex, France	Tricalsium silicate powder, zirconium oxide, water based calcium chloride liquid

4.1.5 Other studied materials

The most important studied materials in all our studies were chlorhexidine (CHX) and in studies III and IV also dimethyl sulfoxide (DMSO) as the final irrigants of root canals before obturation or before post cementation. Other used materials were etchant, adhesives, other irrigants and temporary filling material. All materials were commercially available and are presented in table 4.

Table 4. Other studied materials

Brand	**Type of material**	**Manufacturer**	**Use**	**Study**
ChlorCid	3% sodium hypochlorite	Ultradent, Salt Lake City, UT, USA	1st root canal irrigant	III, IV
EDTA	18% ethylenediamine-tetraacetic acid solution	Ultradent, Salt Lake City, UT, USA	2st root canal irrigant	III, IV
Consepsis	2% chlorhexidine gluconate	Ultradent, Salt Lake City, UT, USA	studied final irrigant	I, II, III, IV
DMSO	5% dimethyl sulfoxide	Sigma-Aldrich, St Louis, MO, USA	studied final irrigant	III, IV
Cavit G	temporary filling material	3M ESPE, Neuss, Germany		III, IV
Artificial saliva	0.7 mmol/l $CaCl_2$ *2 H_2O, 0.2mmo/l $MgCl_2$ * 6 H2O, 4.0 mmol/l KH2PO4, 20 mmol/l Hepes buffer, 0.02 % NaN_3 to prevent microbial growth	Biomedicum Helsinki, Dental laboratory, University of Helsinki, Helsinki, Finland	preservation liquid	I, II, III, IV
Sterile saline		Biomedicum Helsinki, Dental laboratory, University of Helsinki, Helsinki, Finland	final irrigant serving as control	I, II, III, IV

4.2 Methods

4.2.1 Specimen preparation

All roots were separate straight roots of third molars being at least 9 mm long.

Preparation of root canals for post cementation (studies I and II)

Root canals were prepared with Profile (Dentsply-Maillefer, Ballaigues, Switzerland) to size 40/0.04. Apical 1 mm was left unprepared to prevent apical extrusion of irrigants and luting cement.

The round parallel shape of EverStick Post was ensured by shaping it on a glass sheet and light-curing it outside the canal. The post space was made with the post systems own drill with Glassix, D.T. Light-Post and Unicore. The post space of EverStick Post was made with Parapost drill and before cementation Stick Resin was used on the post to activate secondary IPN formation as the manufacturer recommended.

Before cementation the canals were irrigated either with 2% chlorhexidine (test groups) or with sterile saline (control groups). The luting cements were used exactly by the instructions of the manufacturer. A light curing device was used for 40 seconds on the specimen. The light-cure-tip was hold in contact on the post. All teeth were stored in artificial saliva in 37°C for three to seven days to ensure chemical curing of the cements.

Preparation for push-out test (study I, II and IV)

The roots were casted in acryl, the post being vertically positioned, in a Coltoflax (Coltoflax, Colténe/Whaledent, Altstätten, Switzerland) mould. The acrylic rectangle pieces were cut wet with IsoMet Low Speed Saw (Buehler, Lake Bluff, IL, USA) into 2 mm discs (Figure 2). The tapered apical part of the root/post was not included. The diameter of the posts were measured with stereomicroscope (mean value 1.4 mm). Three to four discs per tooth were obtained.

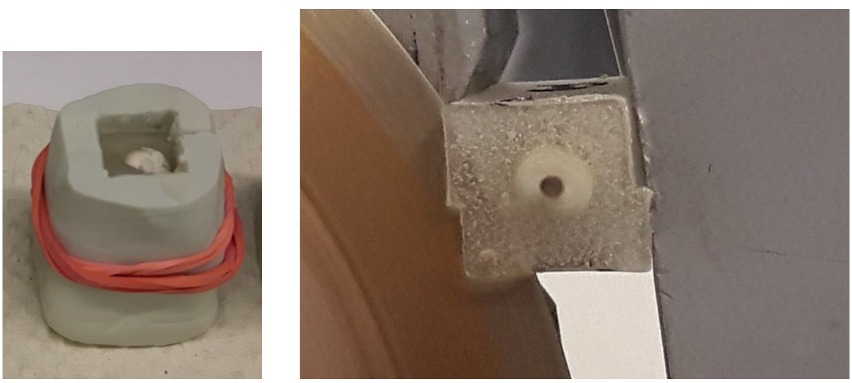


Figure 2. Coltoflax mould and sawing the dentin discs in study IV where the root canals were filled with tricalciumsilicate. Same kind of mould and saw was used with the posts also.

4.2.2 Push-out test

The push-out test was performed by pushing the post from the apical side of each disc using a universal testing machine (Lloyd LRX, Lloyd Instrument Ltd., Fareham, UK) with a custom-made jig and 1.0 mm/min cross-head speed (Figure 3). The force required to debond the post was recorded and the point of failure was observed from the loading curve (Figure 4). Displacement loads (N) were divided by the area of the adhesive interface (area = $2\pi r$ x h, where r is the radius of the disc and h is the thickness of the disc) to determine the bond strength in MPa.

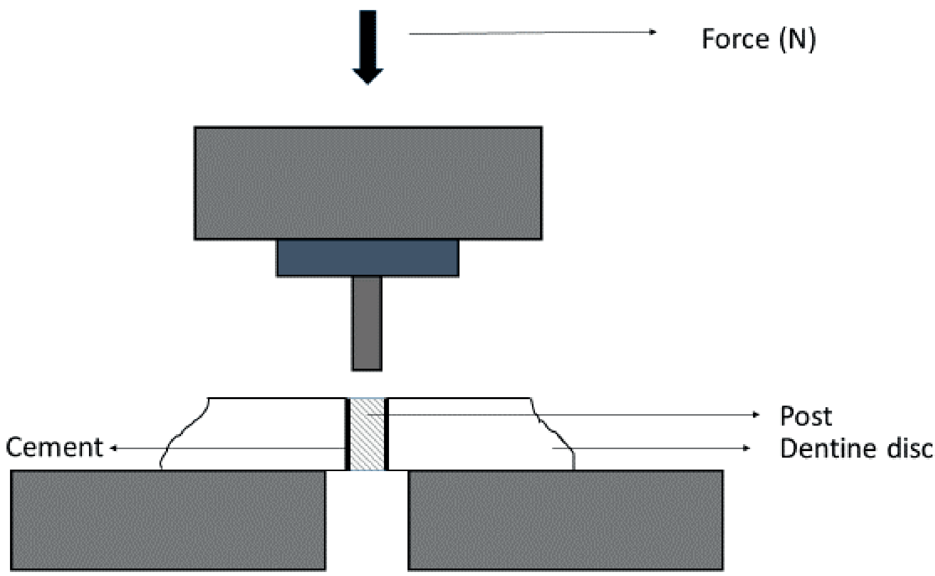


Figure 3. A diagram of the push-out measuring

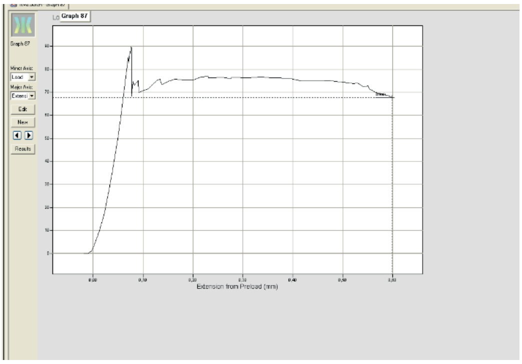


Figure 4. A typical picture of the loading curve where the debonding point could be registered

Preparation and obturation of root canals (study III)

Root canals were prepared with Profile rotary NiTi-instruments (Dentsply Maillefer, Ballaigues, Switzerland) to size 40/0.04. Apical 1 mm was left unprepared to prevent apical extrusion of irrigants and sealer. Between each file size the roots were irrigated with 3% sodium hypochlorite (ChlorCid, Ultradent). After the preparation the canals were irrigated with 2 ml of 3% NaOCl followed by 2 ml of 18% EDTA (Ultradent) to remove the smear layer. In study III the canals of the experimental groups were irrigated either with 5% DMSO (Sigma-Aldrich, St Louis, MO, USA), 2% CHX (Consepsis, Ultradent) or sterile saline (control) for 60 s. Then the obturation was performed using RealSeal SE with RealSeal points and Accessory points M (SybronEndo, Glendora, Canada) or using Topseal with gutta-percha points and gutta-percha auxiliary points C (Dentsply Maillefer). The obturations were performed using lateral condensation technique with one master point and 1-3 accessory points and according to each manufacturer's protocols. The roots were stored in artificial saliva in 37ºC for three days to allow complete setting of the sealers. Artificial saliva was used to ensure the presence of Ca^{2+}– and Zn^{2+} –ions required for the function of dentin endogenous enzymes.

Preparation and filling the root canals (study IV)

The root canals were prepared with Gates burs (Dentsply Maillefer) no 3, 4 and 5 to a final 1.3 mm diameter through the apex. After preparation the roots were irrigated with 3 ml of 3% sodium hypochlorite (ChlorCid, Ultradent) followed by 3 ml of 18% ethylenediaminetetraacetic acid, (EDTA, Ultradent) to remove the smear layer. Before obturation the canals of the experimental groups were irrigated either with 2% CHX (Consepsis, Ultradent) or 5% DMSO (Sigma-Aldrich, St

Louis, MO, USA) for 60 s. The canals of the control groups were irrigated with sterile saline for 60 s. All canals were dried with paper points before obturation. The apex was closed with Coltoflax impression material (Coltoflax, Colténe/Whaledent) during the irrigation. The roots were filled either with ProRoot MTA (Dentsply Maillefer) or Biodentine (Septodont, Saint-Maur-des-Fossés Cedex, France) using amalgam carrier and a plugger, holding the root against a glass sheet, leaving 1 mm of the coronal part of the canal unfilled for temporary filling material (Cavit G, 3M ESPE, Neuss, Germany). The materials were mixed and used according to each manufacturer's protocols. After filling the roots with ProRoot MTA a cotton pellet moistened with saline was placed on the coronal 1 mm of the canal and sealed with Cavit G. The roots filled with Biodentine were left on the table for 12 min to set and then sealed with Cavit G. All samples were stored at 100% humidity at 37ºC until tested.

4.2.3 Fluid filtration test (studies III and IV)

Microleakage was measured by using a fluid filtration method as described by Bouillaguet et al. (Bouillaguet et al. 2008). The apical part of the root was glued with cyanoacrylate glue (Flex Gel, LOCTITE Super Glue, Henkel, Düsseldorf, Germany) into a silicone tube connected to the device recording the fluid flow (Flodec, De Marco Engineering, Geneva, Switzerland). The tube was filled with distilled water under constant hydrostatic pressure of 10 psi (6.89 kPa) (Raina et al. 2007, Wedding et al. 2007). If any tube leakage were observed during the testing, the measurement was stopped, the leaks were sealed and the measurement was repeated. The water pressure was applied to each root for 30 min and the fluid flow was recorded constantly with three seconds time interval (Figure 5).

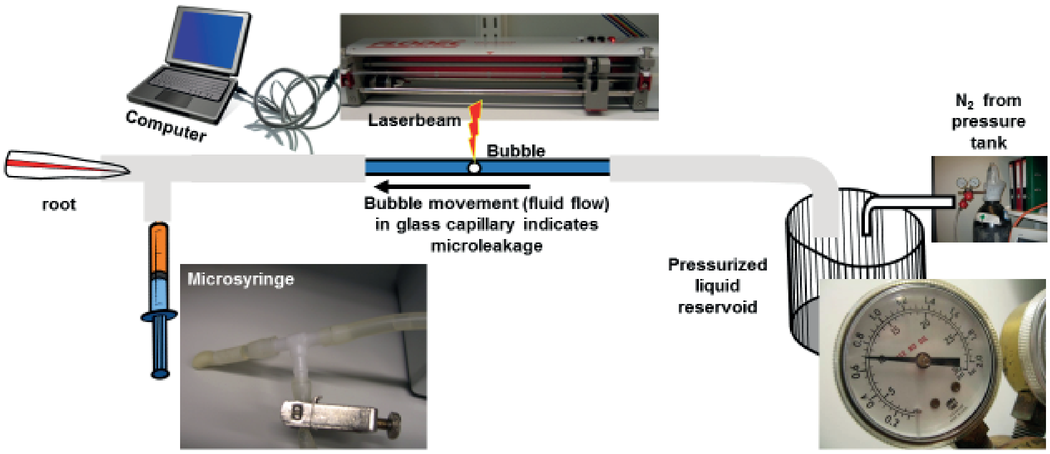


Figure 5. The Flodec fluid filtration system for measuring the microleakage

4.3 Analysis

4.3.1 Fracture mode analyse (studies I, II and IV)

To analyse the fiber post fracture modes, all discs were examined from both sides with a stereomicroscope (Olympus SZ61, Hatagaya, Shibuya-ku, Tokyo, Japan) with 16x magnification, and digitally photographed (Olympus DP 12) from both sides (apical and coronal). After initial examination of all samples, the fractures were classified as follows:

Study I (Figure 6)

a) Mainly cohesive-to-dentin/partially adhesive-to-dentin (CD/AD): The adhesion to dentin was so strong that dentin was fractured and only less than 50% of the fracture area was adhesive
b) Mainly adhesive-to-dentin/partially cohesive-to-dentin (AD/CD): Less than 50% of fracture located in dentin
c) Purely adhesive-to-dentin (AD 100%): The adhesive layer was complete, surrounding the detouched post
d) Mixed failure (Mixed): a combination of all previous modes

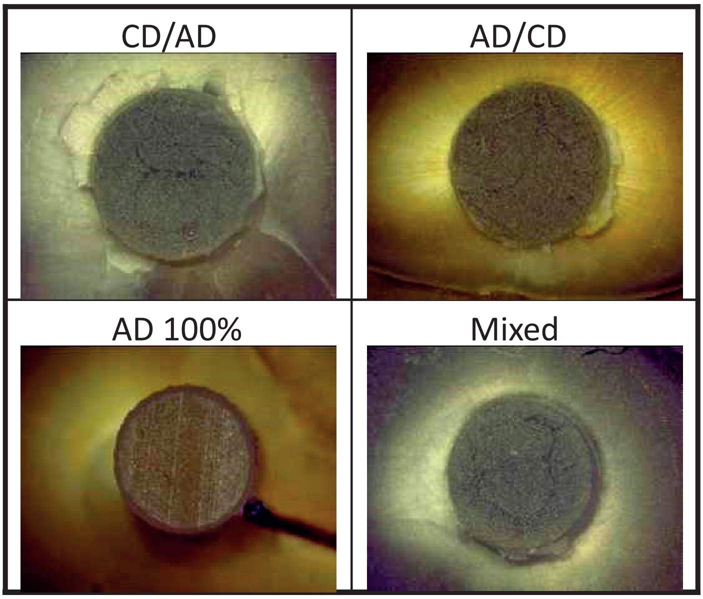


Figure 6. Fiber post fracture types registered in study I. CD/AD: mainly cohesive-to-dentin/partially adhesive-to-dentin. AD/CD: mainly adhesive-to-dentin/partially cohesive-to-dentin. AD 100%: purely adhesive-to-dentin. Mixed: mixed failure (reproduced from Lindblad *et al*. 2010, with permission).

Study II (Figure 7)

a) Pure adhesive-to-dentin failure (AD)
b) Adhesive-to-dentin- and –post failure (AD/AP): the failure was about 50/50 adhesive failure from the dentin and post.
c) Mixed with cohesive-in-cement failure (Mixed/CC): both previously mentioned fracture types along with areas where the cement layer was broken and remnants of cement was found both on post and dentin.
d) Mixed with cohesive-in-dentin failure (Mixed/CD): All modes mentioned above, with cohesive fracture in dentin at some areas.

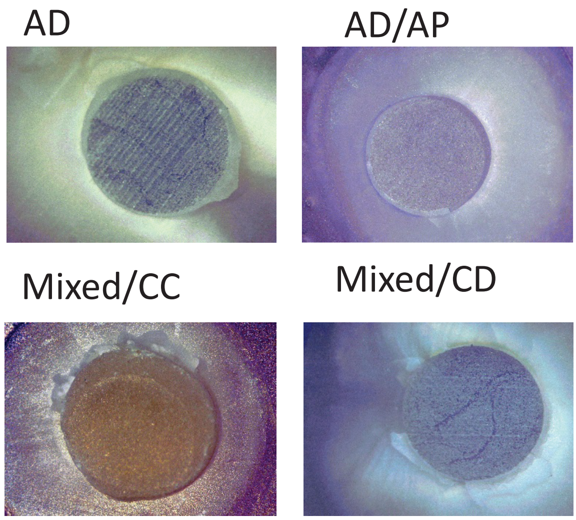


Figure 7. Fiber post fracture types registered in study II. AD: Purely adhesive-to-dentin. AD/AP: Adhesive-to-dentin and adhesive-to-post failure. Mixed/CC: Mixed with cohesive-in-cement failure. Mixed/CD: Mixed with cohesive-in-dentin failure.

Study IV

With hCSCs, only two types of failures were observed. Either the material was broken under the pressure (purely cohesive failure in the tested material), or the material popped out unbroken (purely adhesive failure).

a) Purely cohesive failure in material (Figure 8)
b) Purely adhesive failure (material being unbroken) (Figure 9)

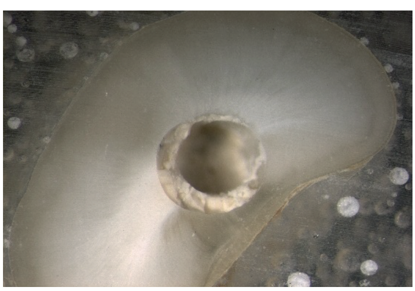


Figure 8. Purely cohesive failure in tested material. On the left apical view and on the right coronal view.

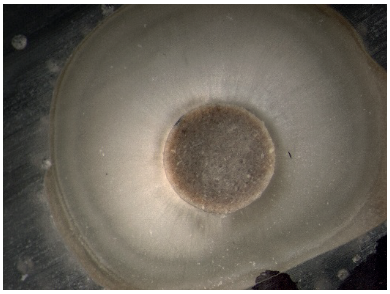


Figure 9. Purely adhesive failure (material being unbroken), coronal view

4.3.3 Statistical analysis

Statistical analysis of all four studies were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). In studies I, II and IV mean push-out bond strength for each root was calculated from the individual discs, and this mean represented the sample. For the failure mode analysis, all discs were analyzed separately. Two-way ANOVA with Tukey´s post-hoc test was used to analyse the differences in bond strength in study I, but as the data did not follow the normal distribution in study II and IV, the Kruskal-Wallis test with Mann-Whitney test was used to analyse the differences in bond strength in these studies. Wilcoxon Signed Ranks test was used to analyse the differences in failure modes between control and CHX–treated samples in each group in studies I and II and to analyse the significance of differences within the groups in study IV.

Kolmogorov-Smirnov and Shapiro-Wilk tests showed that the results of leakage studies III and IV were not normally distributed so the non-parametric methods were chosen for comparison between the groups. Kruskal-Wallis (III, IV) and Mann-Whitney (III) tests were used to analyse the significance of the differences in microleakage between the groups at each time point and Wilcoxon Signed Ranks test was used to analyse the significance of the differences between two time points within the groups (III, IV) and the differences in Vickers hardness values between the material-affected dentin and control dentin in study IV. Pearson Chi-Square test was used to analyse the differences in fracture modes in study IV. The level of statistical significance was set at 0.05.

5. RESULTS

5.1 Effect of CHX on the adhesion of fiber reinforced posts to resin cement and to dentin (studies I, II)

Push-out test (studies I,II)

Initially Unicore and everStickPOST demonstrated significantly higher bond strength values compared to Glassix and D.T. Light-Post, both with and without CHX treatment ($p<0.05$, 2-way ANOVA with Tukey´s post-hoc test) (Fig. 10). When analysed in respect to the adhesive-cement combinations used, PermaFlo DC Primers/PermaFlo DC (used for Unicore) and RelyX Unicem (self-etching cement used for everStickPOST) presented significantly better bond strength than All-Bond 2/Duo-link (used to cement Glassix and D.T. Light-Post) ($p<0.001$, 2-way ANOVA with Tukey´s post-hoc test). With the exception of D.T. Light-Post, CHX slightly (but not statistically significantly) improved the push-out bond strength to dentin.

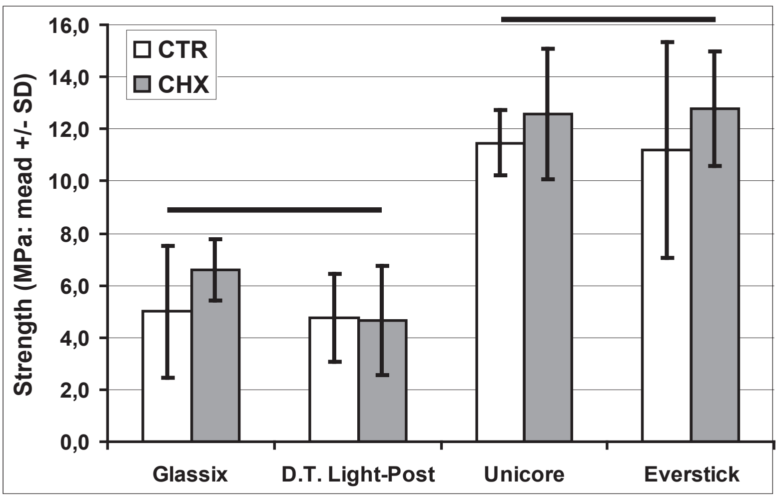


Figure 10. The effect of CHX on the push-out strength with different fiber posts. CTR: control; CHX: chlorhexidine-treated group. Horizontal lines above the bars indicate the groups with no statistically significant differences (reproduced from Lindblad *et al*. 2010, with permission).

After 12 months Unicore with Permaflo DC and everStickPOST with RelyX Unicem still demonstrated significantly higher bond strength values both with and without CHX when compared to respective Glassix and D.T.Light-Post groups cemented with Duo-link ($p<0.05$, Mann-Whitney test) (Figure 11). CHX application did not significantly affect the bond strength after one year of storage in any tested groups, except for D.T.Light-Post/Duo-link, ($p=0.008$) (Figure 11).

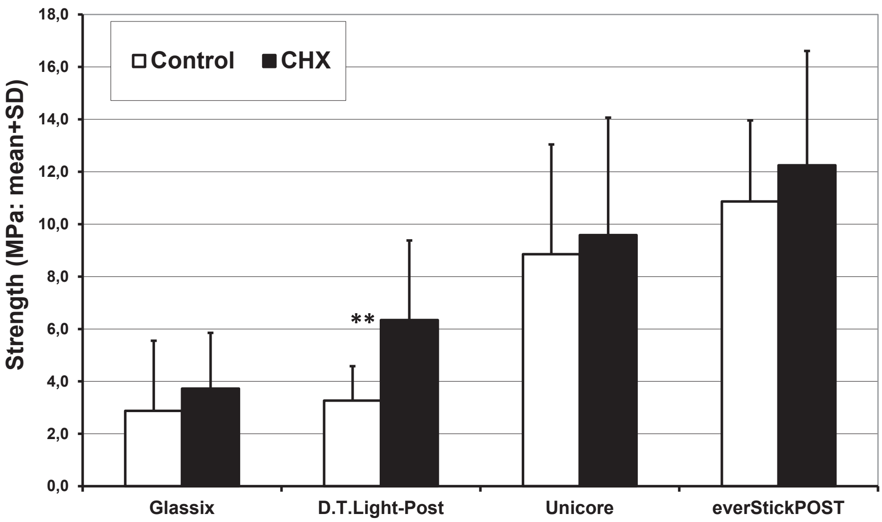
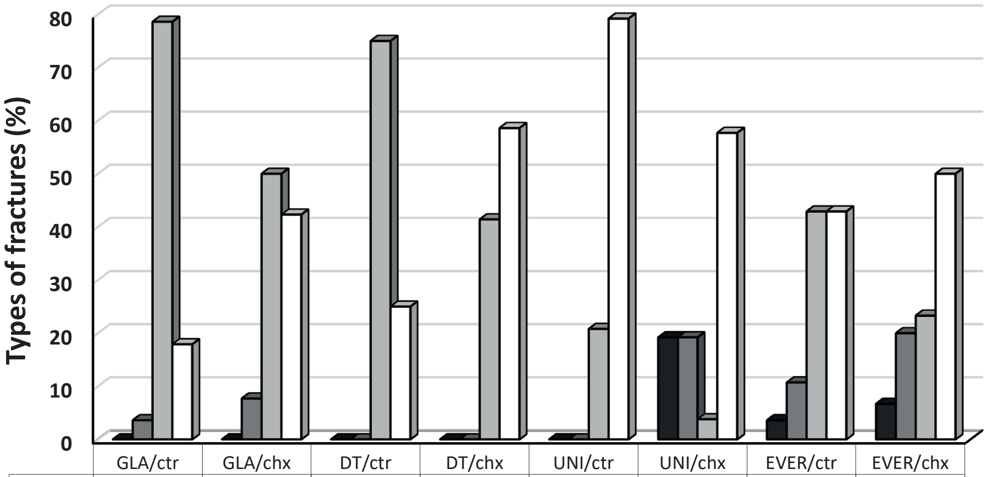


Figure 11. The effect of CHX on the push out bond strength with different fiber posts after one year aging. Control: control group, CHX: chlorhexidine-treated group. **: statistically significant difference ($p<0.05$) between the control and CHX-group with D.T.Light-Post (reproduced from Lindblad *et al*. 2012, with permission).

The effect of CHX to the failure mode immediately and after 12 months (studies I, II)

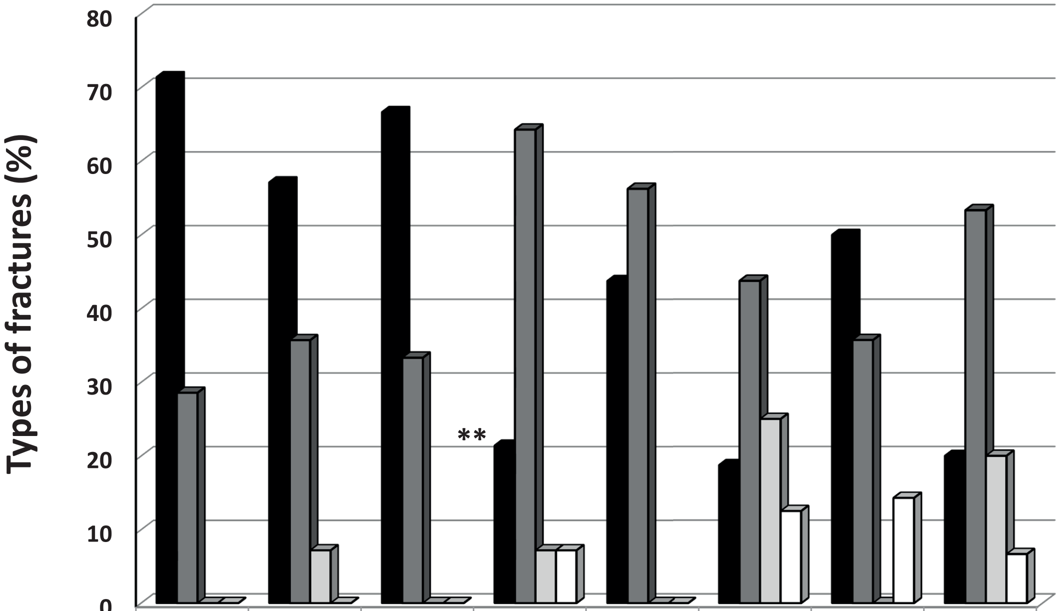
CHX treatment resulted with marked changes in immediate fracture patterns (Figure 12). With CHX, significant reduction of adhesive failures towards dentin cohesive or mixed failures was observed with all posts/cements except with everStickPOST/RelyX Unicem (Wilcoxon Signed Ranks Test) (Figure 12).

Storage for one year CHX changed the fracture pattern with D.T.Light-Post/Duo-link statistically significantly from pure adhesive-to-dentin failure towards mixed and cohesive-in-dentin failures ($p=0.020$) (Figure 13). With other posts, CHX had no effect on the failure modes ($p>0.05$)



	GLA/ctr	GLA/chx	DT/ctr	DT/chx	UNI/ctr	UNI/chx	EVER/ctr	EVER/chx
CD/AD	0	0	0	0	0	19,2	3,6	6,7
AD/CD	3,6	7,7	0	0	0	19,2	10,7	20
AD/CD	78,6	50	75	41,4	20,8	3,8	42,9	23,3
Mixed	17,9	42,3	25	58,6	79,2	57,7	42,9	50

Figure 12. The initial distribution of failure modes (in percentage) between the groups. CD/AD: mainly cohesive-to-dentin/partially adhesive-to-dentin failure type; AD/CD: mainly adhesive-to-dentin/partially cohesive-to-dentin failure type; AD: purely adhesive-to-dentin failure type. Statistical significance in adhesive-to-dentin failures vs. other types of failures between the control and CHX-groups (Wilcoxon Signed Ranks Test, $p < 0.05$) with Glassix post (p=0.02), D.T.Light-Post (p=0.003) and Unicore post (p=0.001). With everStickPOST there was no statistical significance in failure between the control and CHX-groups.



	GLA/ctr	GLA/chx	DT/ctr	DT/chx	UNI/ctr	UNI/chx	EVER/ctr	EVER/chx
AD	71,42	57,14	66,66	21,42	43,75	18,75	50	20
AD/AP	28,57	35,71	33,33	64,28	56,25	43,75	35,71	53,33
mixed/CD	0	7,14	0	7,14	0	25	0	20
mixed	0	0	0	7,14	0	12,5	14,28	6,66

Figure 13. The different types of fractures (in percentage) after one year. AD: pure adhesive-to-dentin failure; AD/AP: adhesive-to-dentin- and -post failure; mixed/CD: mixed with cohesive-in-dentin failure; mixed: mixed failures without cohesive-in-dentin failure; GLA: Glassix post; DT: D.T.Light-Post; UNI: Unicore post; EVER: everStickPOST; ctr: control; chx: chlorhexidine. **: statistically significant difference ($p<0.05$) between the control- and CHX-group with D.T.Light-Post (reproduced from Lindblad *et al*. 2012, with permission).

5.2 The effect of CHX and DMSO on the sealing ability of the tested sealers and calcium silicates with time (studies III, IV)

Leakage test with sealers (study III)

When the effect of irrigant regardless of the sealer was analysed, saline had the highest immediate leakage rate which was significantly higher than with DMSO (p=0.032; Mann-Whitney test). However, the 18-month leakage was significantly lower than the immediate leakage (p=0.027;

related-samples Wilcoxon Signed Ranks test). Since DMSO had a similar low rate of leakage at both time points, 18-month saline controls did not differ from the respective DMSO samples (Figure 14A). CHX immediate values did not differ from the other irrigants, and no change was observed when the immediate and 18-month CHX samples were compared. However, the 18-month leakage with CHX was significantly higher than with saline or DMSO ($p=0.002$ and $p=0.001$, respectively; Mann-Whitney test) (Figure 14A).

When the effect of the sealers regardless of the irrigant was analysed, Topseal had higher immediate leakage rate than RealSeal SE ($P=0.09$; Mann-Whitney test). At 18 months, there was no difference in leakage between the sealers (Figure 14B).

Effect of irrigants within the sealers

Next, the effect of irrigants and sealers were analysed together. In immediate testing, even though RealSeal SE in general performed better than Topseal in all groups, the difference between the sealers was statistically significant only in CHX-irrigated group ($p=0.035$; Mann-Whitney test) (Table 6, Figure 15A). DMSO irrigation produced the lowest mean microleakage for both sealers, followed by CHX and control groups. However, within sealers, the differences were not statistically significant.

When 18 months aged groups' microleakage were compared to the immediate values, slightly lower values were found in control and DMSO-irrigated groups and slightly higher values in CHX-irrigated groups for both RealSeal SE and Topseal. However, the differences were not statistically significant in any of the groups (Table 6).

Also after 18 months aging, RealSeal SE demonstrated less microleakage than Topseal in all the groups, but the differences were not statistically significant within any irrigation group (Table 6, Figure 15B). For both sealers DMSO had the lowest mean microleakage values, which were also statistically significantly lower than with CHX irrigation within sealers ($p<0.009$ for Topseal and $p=0.04$ for RealSeal SE; Mann-Whitney test). Two RealSeal SE samples had practically no leakage at all (less than 0.01 nl/min). For both sealers, final irrigation with CHX had the highest microleakage scores, and with RealSeal SE the microleakage with CHX was significantly higher than in controls ($p=0.022$; Mann-Whitney test). The microleakage values between the DMSO-irrigated and controls did not reach statistical significances.

		Immediate					
		Mean	SD	Median	Min	Max	95% CI of mean
RealSeal	**Control**	724.5^{AB}	384.7	798.1	152.0	1222.7	428.8; 1020.2
	CHX	647.3^{A}	464.6	544.4	162.0	1734.5	290.1; 1004.4
	DMSO	448.0^{A}	180.5	405.4	243.5	718.3	309.2; 586.8
TopSeal	**Control**	1316.7^{B}	879.5	1183.6	185.8	3191.7	687.0; 1945.9
	CHX	1080.3^{B}	300.5	961.2	773.2	1686.8	802.4; 1358.1
	DMSO	687.3^{AB}	571.9	471.0	140.9	1717.5	244.7; 1126.9
		18 months					
		Mean	SD	Median	Min	Max	95% CI of mean
RealSeal	**Control**	460.8^{A}	215.1	565.1	233.2	746.3	295.5; 626.1
	CHX	747.4^{BC}	264.1	723.3	475.9	1369.9	544.4; 950.5
	DMSO	403.6^{A}	309.0	348.5	0.0	823.2	166.1; 641.1
TopSeal	**Control**	665.2^{ABC}	411.4	575.0	102.7	1491.7	370.9; 959.6
	CHX	1173.5^{C}	440.9	996.1	762.1	2069.8	765.7; 1581.3
	DMSO	526.3^{AB}	251.7	559.5	6.9	789.7	332.9; 719.8

Table 6. Microleakage (nl/30 min) of the samples. The group mean values with different superscript letter demonstrate statistically significant differences within each time point (reproduced from Lindblad *et al*. 2019, with permission).

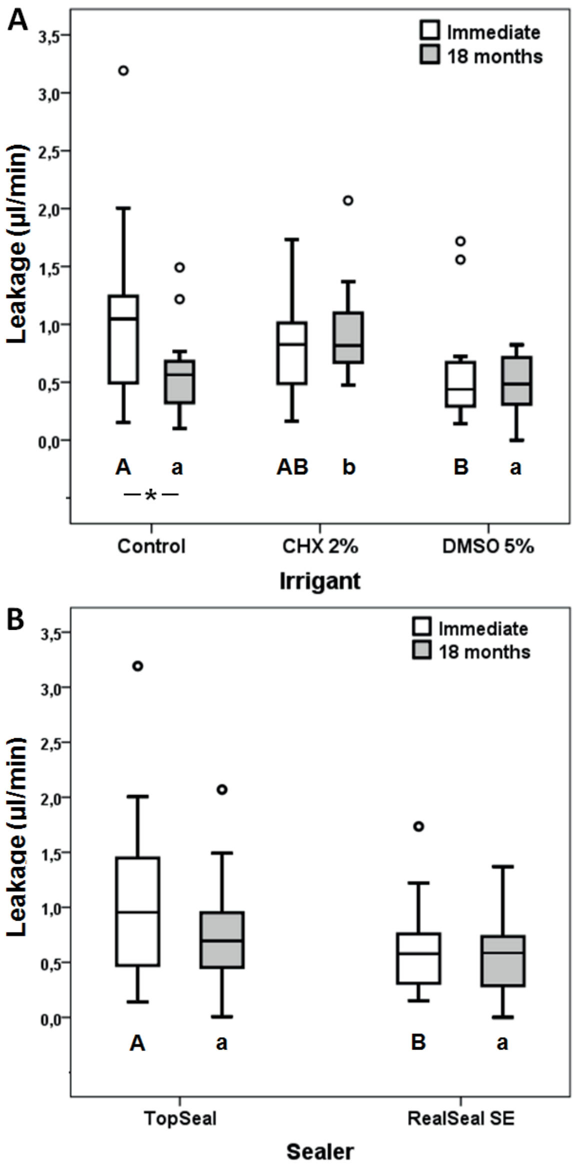
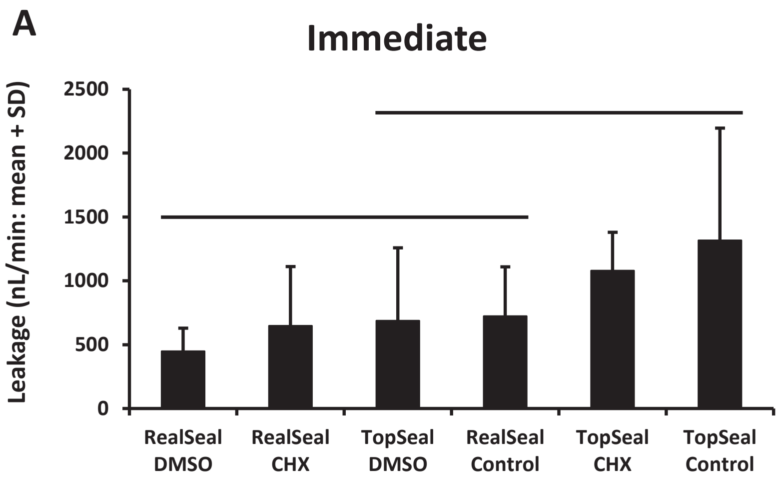


Figure 14. A) The effect of irrigant regardless of the sealer on the microleakage. Upper and lower case letters indicate the statistically significant difference between the irrigants at the immedieate and 18-month measurements, respectively. B) The effect of the sealer regardless of the final irrigant. The letters indicating the statistical significance are the same as in Figure 12A. The circles indicate extreme values identified by the statistical program (reproduced from Lindblad *et al*. 2019, with permission).



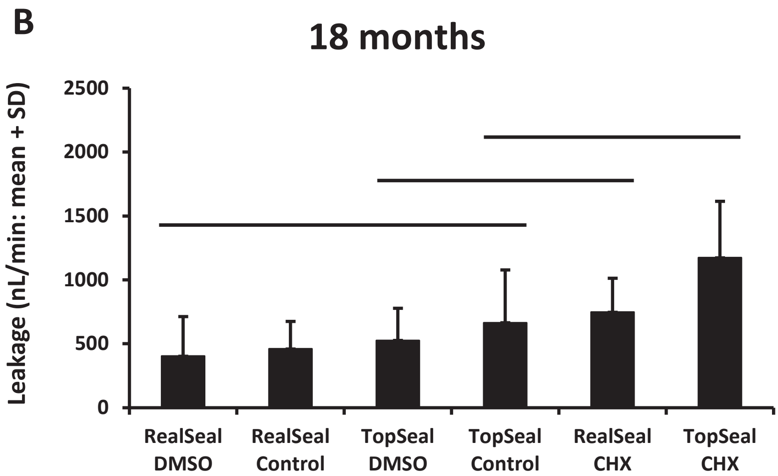


Figure 15. The immediate (A) and 18-month aged (B) microleakage for each group, presented in ascending order. The bars connected with horizontal lines did not show statistically significant differences (reproduced from Lindblad *et al*. 2019, with permission).

Microleakage with calcium silicates immediately and after six months (study IV)

The immediate and 6-month leakage values are presented in Figure 16. The immediate leakage was low (between 394 and 681 nl/30 min) in all groups, with no significant differences between the groups. The leakage increased significantly during the 6-month storage compared to the immediate values in all groups except in Biodentine-CHX group and Biodentine-DMSO group. The lowest leakage after 6 months was observed in the Biodentine-DMSO group, which was comparable to the ProRoot MTA-control group ($p>0.05$). Even though both CHX and DMSO irrigation significantly increased the leakage with ProRoot MTA with time, there was no statistically significant difference compared to the ProRoot MTA-control group at six months' time point, mainly due to high variation in leakage in both experimental irrigant groups.

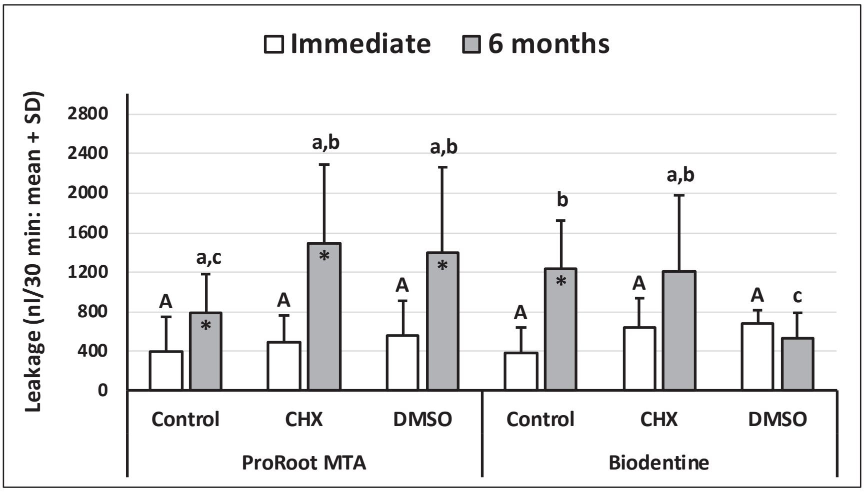


Figure 16. Leakage values (mean + SD) in different groups and time points. Different upper case letters indicate statistically significant differences between the groups at the immediate testing, and lower case letters at the 6-month testing (Kruskal-Wallis and Mann-Whitney tests, $p < 0.05$). * indicates statistically significant difference from the immediate testing within the group (Wilcoxon signed rank test, $p<0.05$).

5.3 The effect of CHX and DMSO on adhesion of calcium silicates to dentin (study IV)

Push-out test with calcium silicates (study IV)

The 6-month push-out strength values are presented in Figure 17. ProRoot MTA with DMSO as the final irrigant and ProRoot MTA control showed the highest 6-month push-out bond strength, which was significantly reduced with CHX irrigation. With Biodentine, irrigation with CHX or DMSO resulted with significantly higher push-out strength compared to the Biodentine control group.

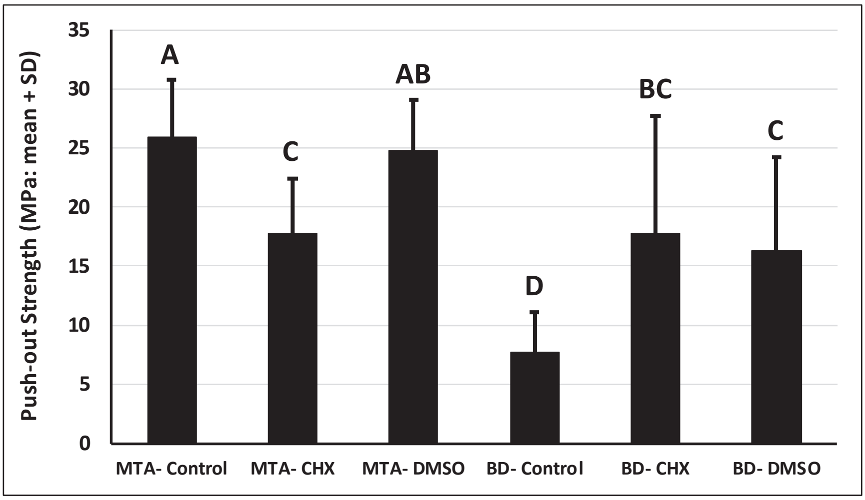


Figure 17. Push-out strength (mean + SD) in different groups. Different upper case letters indicate statistically significant differences between the groups (Kruskal-Wallis and Mann-Whitney tests, $p < 0.05$).

Fracture analysis of calcium silicates (study IV)

Fracture modes are presented in Figure 18. There was a statistically significant difference in the distribution of the fractures between the groups (Pearson Chi-Square test, $p<0.05$). With both ProRoot MTA and Biodentine, DMSO irrigation slightly reduced the purely adhesive fractures and increased the cohesive dentin fracture component. On the contrary, CHX irrigation increased the purely adhesive fractures both with ProRoot MTA and Biodentine (by 26% and 38%, respectively) compared to the control group.

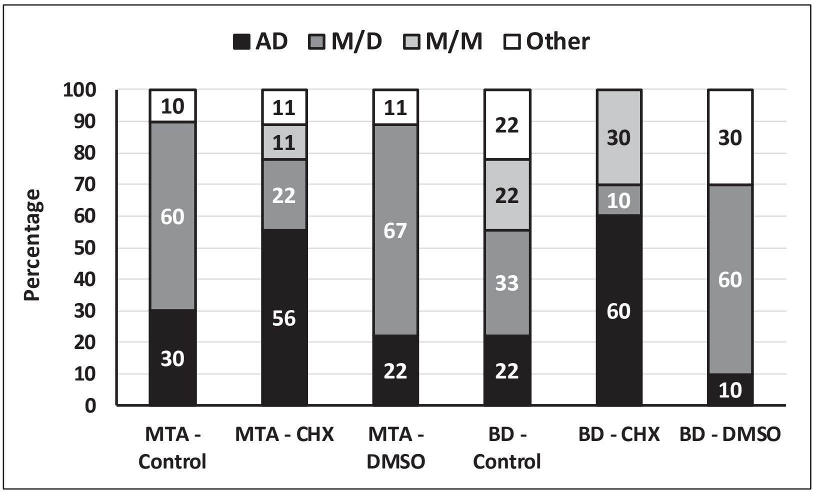


Figure 18. Push-out fracture mode percentages in different groups. AD: purely adhesive fracture; M/D: mixed fracture with adhesive and cohesive in dentin; M/M: mixed fracture with adhesive and cohesive in material.

There was no correlation between the root canal leakage and push-out bond strength when the whole data was included (Figure 19). The only subgroup to show statistically significant correlation was the MTA-DMSO group (Spearman correlation coefficient -0,783, p=0.013).

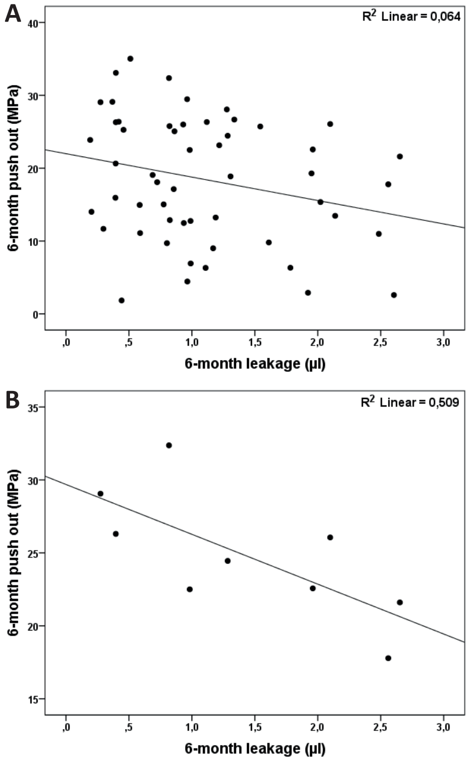


Figure 19. Correlation between the leakage and push-out bond strength **A)** for the whole data (Spearman correlation coefficient -0.236, p = 0.090) and **B)** for the MTA-DMSO group that was the only group with statistically significant correlation (Spearman correlation coefficient -0,783, p = 0.013).

5.4 The effect of CHX and DMSO with calcium silicates on dentin microhardness (study IV)

The dentin microhardness (Vickers hardness, VHN) values are presented in Figure 20. In all groups except Biodentine-DMSO the dentin immediately under the interface was significantly harder than in the control area of the same tooth. ProRoot MTA with (127.1 ± 8.1%) and without (120.4 ± 4.1%) DMSO as the final irrigant showed significantly higher dentin hardness than any Biodentine-group. ProRoot MTA with CHX irrigation (123.0 ± 12.6%) did not show statistically significant difference to any other group except to Biodentine DMSO group (100.8 ± 10.4%), which was significantly lower than in any other group.

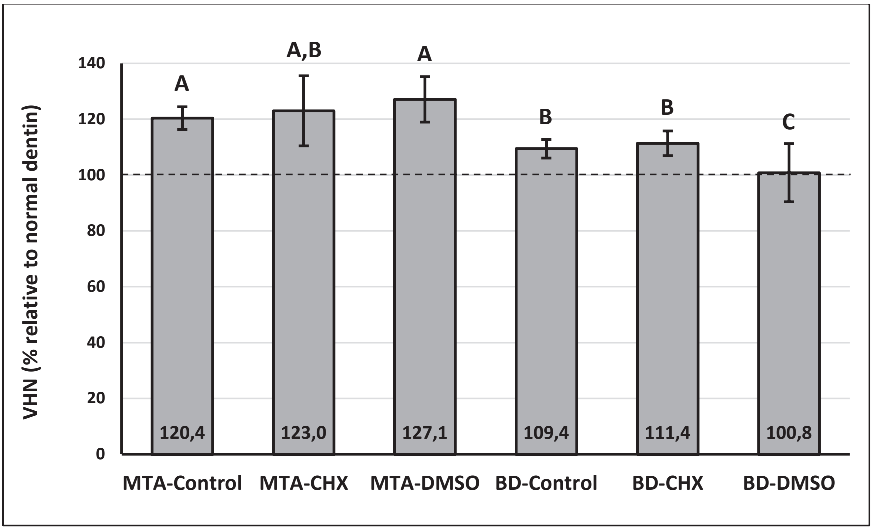


Figure 20. The effect of the obturation materials and irrigants on dentin microhardness (VHN) immediately below the dentin-root canal border. The bars indicate the relative hardness (mean ± SD) compared to the respective normal dentin within the sample (dashed line: intrinsic control, 100%). The values at the base of the bars present the mean for each group. Different upper case letters indicate statistically significant differences between the groups (Kruskal-Wallis and Mann-Whitney tests, $p < 0.05$).

6. DISCUSSION

6.1 General discussion

In reparative dentistry root canal treatment is a common procedure due to the bacterial invasion in the root canal or the need for more support to the crown restauration. It is still a challenge to get a tight and long lasting adhesion between the root canal dentin and the material used in the root canal (luting cement, calcium based cement, endodontic sealer). The major cause to lose the restauration is the failure of adhesion and leakage between dentin and the material. The adhesion between the bonding agent and composite is a chemical process, where the double bonds of carbon chain polymerize. The adhesion to enamel and dentin is mainly micromechanical. In enamel the resin of the bonding agent is attached to the acid etched porous enamel. In dentin the resin penetrates between the collagen fibers exposed by acid forming the hybrid layer. On the other hand some self-etching adhesives that have low pH, can form a bond between acidic monomers and calcium from dentin hydroxyapathite (Van Landuyt et al.2010. However this bonding is very liable to various mechanisms that can weaken the bond immediately or with time. One known factor are the enzymes that degrade the collagen fibers in the hybrid layer. This series of *in vitro* studies aimed to evaluate the effect of two irrigants that can inhibit the collagen degradation (CHX, DMSO) and by that could have a positive influence in preserving the adhesion to dentin.

At first the study was carried out with CHX, which is widely used in endodontics for its good antimicrobial properties (Basrani et al. 2002) and is in general thought not to affect negatively the adhesion to root canal dentin as NaOCl may do (Barreto et al. 2016, Ozturk et al. 2004). CHX also improves the longevity of composite adhesive bonding to coronal dentin (Carrilho et al. 2007b, Carrilho et al. 2007a). After the first studies new research from the effects of DMSO to the dentin wettability and possible ability to MMP inhibition (Mehtälä et al. 2017, Tjäderhane et al. 2013a, Mehtälä et al. 2010) woke the interest of testing also this irrigant in addition to CHX. DMSO could have a more extensive use in dentistry in the future.

The effect of these two irrigants on the adhesion to dentin in the root canal was investigated with push-out test (study I, II and IV) and with microleakage measurements (III, IV). With calcium silicate cements the possible influence of these cements on dentin was also investigated.

6.1.1 Bonding properties

Push-out test

The push-out test has for many years been valued as a suitable and reliable test for testing the bond strength and adhesion of different materials in root canal (Goracci et al. 2004). The specimen failure rate with this test is low. The interfacial bond strength is determined by recording the force required for the debonding. The push-out bond strength is then calculated from the displacement load taking into consideration the area of the adhesive interface.

The push-out testing method has been argued to have some limitation though. For example different pin size, composition/stiffness of the tested material and different root canal diameter could affect the results (Pane et al. 2013, Chen et al. 2013). Pane et al found that the bond strength was lower when the pin was 50%-60% of the canal size. In our study this problem was taken care of by using a pin with a diameter only 0,1mm smaller than the diameter of the tested material. The root canal diameter and also the diameter of the material was standardized in this study. With fiber posts the density of the fibers and the filler size between the fibers could of course affect the results, but this was taken into account when the failure modes were observed. With tricalcium silicate cements the difference in firmness of the material might at least partly affect the push-out measurements. This was observed also in the failure modes as we found more cohesive fractures in cement in Biodentine than in Pro-Root MTA groups (Study IV). It is also very important that the angle of the discs relative to the post in root canal or the long axis of root canal is constant (90º post orientation). With root canal posts this problem was easier to take care of than with calcium silicate cements. One criteria on the roots to all our studies was that they were straight by visual observation. All canals of the roots tested with push-out test were also drilled to get a straight canal space.

Previous studies have shown that CHX does not affect the immediate composite resin bond strength to dentin but increases the long term bond strength to coronal dentin (Carrilho et al. 2007a, Carrilho et al. 2007b, Soares et al. 2008a, Soares et al. 2008b, de Castro et al. 2003). The present results of the push-out bond strength, when CHX was used with fiber-reinforced posts cemented with luting cements, are in concert with these. However the possible increase of even the immediate bond strength (although not significant) could be seen with the change of failure mode. The number of pure adhesive failures decreased and dentin cohesive failures increased with CHX. The same pattern of failure modes could be seen with CHX after one year. In addition to the increased cohesive-to-dentin failures, with CHX we could also see cohesive failures in the cement, which in immediate testing were not found at all. The change in failure pattern indicates that CHX may have prevented the degradation of the hybrid layer even though the improvement could not be seen in the push-out bond strength testing, since the mechanical properties of dentin, the cements, or bond strength to the post were decreased. In general, the combination Unicore post/PermaFlo DC and everStickPOST/RelyX Unicem showed higher bond strength both immediately and after one year regardless whether CHX was used or not. The good results with PermaFloDC are in concert with other studies (570 Burgin et al.2017, Bitter et al. 2004) in contrary to many studies of RelyXUnicem. The differences with the results of RelyXUnicem may partly depend on the different testing methods and different post used (Hiraishi et al. 2009, Holderegger et al. 2008, Mazzoni et al. 2009, Das et al. 2015, Bitter et al. 2006). EverStickPOST, cemented with RelyXUnicem after dimethacrylate resin treatment on the post, possibly became more compatible with the resin system in RelyX Unicem cement, and enhanced secondary IPN type of bonding (Vallittu 2009). In the formation of secondary IPN bonding, the semi-IPN polymer matrix of the EverStickPOST is affected by the monomers of the dimethacrylate adhesive resin which dissolves and swells the surface of the post. Once the resins have become polymerized, the secondary IPN bonding has been formed (Vallittu 2018b). As conclusion of the effect of CHX on the push-out bond strength immediately and after one year, the bond strength preserving effect of CHX could not be seen as clearly with post bonding as it has shown to be with composite fillings in crown dentin.

The effect of CHX on the push out strength of tricalcium silicate cements (Study IV) differed from the effect on post bonding. CHX significantly reduced ProRoot MTAs bond strength which is in concert with some other studies (Elnaghy et al. 2014, Guneser et al. 2013, Hong et al. 2010, Holt et al. 2007). Hong et al supposed that CHX disturbs the setting of ProRoot MTA. In their study the typical crystal structure on the surface of the MTA was not seen when CHX was in contact with ProRoot MTA before initial setting (Hong et al.2010). Biodentine showed lower push-out bond strength than ProRootMTA, but CHX increased significantly the bond strength of Biodentine. The reason for this is not known. With both calcium silicate cements the amount of adhesive failures increased with CHX, so the actual effect of CHX on the adhesion of tricalcium silicate cements still remains unclear.

DMSO increased the push-out bond strength with both cements and decreased the amount of pure adhesive failures. One reason for this could be the increased wettability by DMSO. Since DMSO changes the behavior of water (Vishnyakov et al.2001) and changes in hydration may affect tricalcium silicate properties (Koutroulis et al. 2019), DMSO effects may also be related to the availability of water molecules at the dentin-material interface.

Leakage test

Measuring the leakage in dentistry is very challenging. Many different methods have been used: fluid filtration, dye penetration, glucose penetration, bacteria and toxin infiltration, protein microleakage test (bovine albumin and caffeine), radioisotope penetration, etc (Jafari et al. 2017). The attitude to leakage studies is contradictory, which is understandable. There are numerous factors that can affect the results: molecule size of the used subject, the pressure used during testing, the time used for observation, to mention a few. However, when measuring the leakage, we could at least see the possible changes within time and compare the leakage and the materials used in the same study. The superiority of one material to another is of course not possible to be determined by measuring the leakage only. The fluid filtration method was chosen to analyze the sealing ability of two sealers and the adhesion of calcium silicate cements to root dentin because we wanted to see the time-related effect of CHX and DMSO on the adhesion of these materials also within time (Study III and IV). One benefit of this non-destructive method is that the specimen could be measured again (Verissimo et al. 2006, Miletic et al. 2002). Another benefit with this method is that the quantitative data is collected by the computer to minimize the operator error. When measuring the same samples again after aging (study IV), the change during time could be seen, and not just the sealing ability of two materials were compared to each other in one time point. In study IV also other methods were used to get a wider understanding of the tested materials.

Neither CHX nor DMSO affected the immediate sealing capacity of the two tested sealers (RealSeal SE and Topseal). After 18 months the microleakage did not increase. However, both sealers with final irrigation with CHX showed the highest microleakage scores, and with RealSeal SE the microleakage with CHX was significantly higher than in controls. These findings indicate that inhibition effect of CHX on MMPs does not have a similar role with sealers as it has with adhesives. Since EDTA, which was used before the experimental irrigants, is a chelator and exposes the collagen in dentin surface, the degradation of the exposed collagen by endogenous enzymes present also in root dentin (Santos et al. 2009, Tay et al. 2006) is expected. The reason may be the difference between the natures of the interface. Composite adhesive bonding, including self-etch adhesives, relies on the penetration of the primer/adhesive monomers between the exposed collagen fibers and the mechanical interlocking of collagen and polymerized adhesive (Pashley et al. 2011a, Van Meerbeek et al. 2011), while the sealers rely on more mechanical interlocking with the dentin surface and dentinal tubule penetration. Final irrigation with DMSO resulted with the lowest microleakage. Previous studies have shown that DMSO improves wettability by increasing the surface free energy and by that it might also improve the adhesion (Ballal et al. 2013). On the other hand, CHX has also been shown to improve wettability (Ferreira de Assis et al. 2011), but the microleakage was highest with CHX with both sealers. These findings do not indicate that simply increasing the wettability the sealing ability could be improved.

One explanation for the higher microleakage with CHX could be the precipitate formed when 0,2% - 2% CHX (di)gluconate is used after EDTA (Rossi-Fedele et al. 2012). This white salt is formed by neutralization of the cationic CHX by anionic EDTA (Rossi-Fedele et al. 2012). Vivacqua-Gomes et al. have shown that the precipitate resulting from NaOCl and 2% CHX gluconate causes significantly higher leakage than the other studied groups (Vivacqua-Gomeset al. 2002). Although the chemical composition of the precipitate between NaOCl and CHX is different from that of EDTA and CHX, this precipitate formation could be detrimental to the adhesion and sealing ability of the root filling and the chemical degradation of CHX caused by this reaction may also disturb the enzyme inhibition effect of CHX. Krishnamurthy et al. have shown that the precipitate formed between NaOCl and CHX could be prevented by using absolute alcohol between the two irrigants (Krishnamurthy et al. 2010). Maybe the use of CHX + ethanol after EDTA could show different leakage results.

When studying the leakage of root canals filled with calcium silicate cements (Study IV), the results are in concert with the sealer study. CHX showed a little higher microleakage than DMSO after six months with both hCSC's, being statistically significantly higher only with Biodentine. In study IV DMSO decreased the microleakage statistically significantly only with Biodentine. Although DMSO (Mehtälä et al.2017) (and also to minor extend CHX (Prado et al. 2011) increase dentin wettability and water is vital for hCSCs from the setting to their bonding and capability of forming new hydroxyapatite with root canal dentin (Prati et al. 2015, Camilleri 2015), DMSO-induced preservation of the interface integrity was seen only with Biodentine-DMSO group. These findings indicate that leakage-free integrity of the interface between hCSC and root dentin is not achievable just by increasing the wettability. The effect of the salt formed between EDTA and CHX on the setting of hCSC's is not known.

6.2 Biomineralization

Surface microhardness

The effect of the tested material on dentin surface microhardness values (VHN) was tested with Vickers hardness test (VHN). The dentin at the interface of the material was significantly harder than the control dentin in every group except with Biodentine-DMSO. This finding is in accordance with a recent study by Cardoso et al. (Santos et al. 2018). With ProRoot MTA-control and ProRoot MTA-DMSO the hardness of dentin was significantly higher than with any Biodentine group, which indicates that the ability to increase the biomineralization is higher with ProRoot MTA. Our findings are in concert with some other studies, although similar studies of the effect of hCSC's on dentin hardness are not found. Hinata et al. found high biomineralization ability of Pro-Root MTA in their study (Hinata et al. 2017). In another study, the ability of forming new reparative dentin was higher with Pro-Root MTA than with Biodentine although the difference was not statistically significant (Kim et al. 2016). Since our results in the leakage study showed slightly opposite results, we cannot assume that leakage would decrease only by remineralization as was suggested by Reyes-Carmona et al. (Reyes-Carmona et al. 2009, Reyes-Carmona et al. 2010). EDTA (used also in our study) reduces the smear layer and exposes the dentinal tubules. Previous studies have shown that hCSCs can permeate into the tubules. The excess calcium, silica and phosphate of hCSCs induce intratubular mineralization, also called biomineralization. In biomineralization the precipitated amorphous calcium phosphate form crystallized hydroxyl apatite that penetrate into the dentinal tubules (Valimaa et al. 20189. This remineralization reaction of dentin cause occlusion of the dentinal tubules (Valimaa et al. 2018, Dong et al. 2011). Another factor that cause dentinal tubules occlusion is sclerosis. Thaler et al. showed that permeability of dentin was strongly influenced by age of the patient and location in root (Thaler 2008). Dentin sclerosis affects the diameter of dentinal tubules, which may have an influence on the penetration ability of medicals into the tubules (Paque et al. 2006) and by that also on the dentin hardness. In the present study the age of the patient was not known, but the location was taken into account by using the same level of root dentin disc of all tested specimen.

7. Clinical considerations and future investigations

Adhesion of different kind of materials to the root canal and root dentin is an important part of restorative dentistry. The prognosis of endodontics depend not only on the healing of the infection but also the inhibition of reinfection. This requires tight and time preservative seal between the endodontic material and root dentin. Another challenge besides the obturation is the restorative materials and their adhesion to the root dentin. The coronal filling may need extra support from the root canal, where the post bonding is crucial for the coronal seal. The costs for root canal treatment and/or restauration with root canal post are quite high and the result should serve the patient as long as possible without the failure. As shown above, different testing methods can be used to compare different materials, but one testing method just allow us to compare some properties of these materials over time. Care should be taken when interpreting experimental data and their clinical relevance. For example, chlorhexidine has been shown to enhance the adhesion of coronal filling to coronal dentin. In root dentin the same kind of effect may occur with composite resin cements, but with the root canal sealers and hCSC's the similar positive effect in microleakage could not be seen. On the other hand, chlorhexidine has good antimicrobial properties that could be crucial to the healing of the periapical and intracanal infection. In addition, irrigation with 2% CHX after NaOCl and EDTA enhance the fracture resistance of roots filled with AH Plus (Turk et al. 2017). However as 2 % CHX had the worst long term results with both sealers, the use of it as final irrigant should perhaps be reconsidered in clinical setting. The optimal balance between the good antibacterial effect and the potentially harmful effect on sealing ability with CHX still needs further research.

The use of DMSO as the final irrigant demonstrated positive effects with every testing method, although not all results were statistically significant. DMSO with its wettability enhancing feature could be useful in endodontics, but needs further research.

In conclusion, 100% tight seal or adhesion of the tested materials to the root canal dentin seems not to be possible to achieve. Even the calcium silicate-based materials with their presumed biomineralization of the interface fail to improve the seal with time. Further research should concentrate to develop materials that allow reproducible creation of impermeable root canal obturations. Also the bonding characteristics and the inhibition of the degradative enzymatic activity in root canal needs better understanding and further research.

8. REFERENCES

Akcay H, Arslan H, Akcay M, Mese M, Sahin NN. Evaluation of the bond strength of root-end placed mineral trioxide aggregate and Biodentine in the absence/presence of blood contamination. Eur J Dent 2016;10:370-375.

Aksornmuang J, Nakajima M, Foxton RM, Tagami J. Mechanical properties and bond strength of dual-cure resin composites to root canal dentin. Dent Mater 2007;23:226-234.

Al-Assaf K, Chakmakchi M, Palaghias G, Karanika-Kouma A, Eliades G. Interfacial characteristics of adhesive luting resins and composites with dentine. Dent Mater 2007;23:829-839.

Anchieta RB, Machado LS, Martini AP, Santos PH, Giannini M, Janal M, Tovar N, Sundfeld RH, Rocha EP, Coelho PG. Effect of long-term storage on nanomechanical and morphological properties of dentin-adhesive interfaces. Dent Mater 2015;31:141-153.

Angeloni V, Mazzoni A, Marchesi G, Cadenaro M, Comba A, Maravi T, Scotti N, Pashley DH, Tay FR, Breschi L. Role of Chlorhexidine on Long-term Bond Strength of Self-adhesive Composite Cements to Intraradicular Dentin. J Adhes Dent 2017;19(4):341-348.

Arrais CA, Giannini M, Rueggeberg FA, Pashley DH. Microtensile bond strength of dual-polymerizing cementing systems to dentin using different polymerizing modes. J Prosthet Dent 2007;97:99-106.

Asmussen E, Peutzfeldt A, Heitmann T. Stifness, elastic limits and strength of newer types of endodontic posts. J Dent 1999;27:275-278.

Atmeh AR, Chong EZ, Richard G, Festy F, Watson TF. Dentin-cement interfacial interaction: calcium silicates and polyalkenoates. J Dent Res 2012;91:454-459.

Babb BR, Loushine RJ, Bryan TE, Ames JM, Causey BS, Kim J, Kim YK, Weller RN, Pashley DH, Tay FR. Bonding of self-adhesive (self-etching) root canal sealers to radicular dentin. J Endod 2009;4:578-582.

Ballal NV, Tweeny A, Khechen K, Prabhu KN, Satyanarayan, Tay FR. Wettability of root canal sealers on intraradicular dentine treated with different irrigating solutions. J Dent 2013;41:556-560.

Barfeie A, Thomas MB, Watts A, Rees J. Failure Mechanisms of Fibre Posts: A Literature Review. Eur J Prosthodont Restor Dent 2015;23:115-127.

Barreto MS, Rosa RA, Seballos VG, Machado E, Valandro LF, Kaizer OB*, So M Bier C.* Effect of Intracanal Irrigants on Bond Strength of Fiber Posts Cemented With a Self-adhesive Resin Cement. Oper Dent 2016;41:e159-e167.

Basrani B, Lemonie C. Chlorhexidine gluconate. Aust Endod J 2005;31:48-52.

Basrani B, Santos JM, Tjäderhane L, Grand H, Gorduysus O, Huang J, Lawrence HP, Friedman S. Substantive antimicrobial activity in chlorhexidine-treated human root dentin. Oral surg Oral Med Oral Pathol Oral Radiol Endod 2002;94(2):240-245.

Bergmans L, Moisiadis P, De Munck J, Van Meerbeek B, Lambrechts P. Effect of polymerization shrinkage on the sealing capacity of resin fillers for endodontic use. J Adhes Dent 2005;7:321-329.

Bhavana V, Chaitanya KP, Gandi P, Patil J, Dola B, Reddy RB. Evaluation of antibacterial and antifungal activity of new calcium-based cement (Biodentine) compared to MTA and glass ionomer cement. Journal of Conservative Dentistry 2015;18:44-46.

Bitter K, Meyer-Lueckel H, Priehn K, Kanjuparambil JP, Neumann K, Kielbassa AM. Effects of luting agent and thermocycling on bond strengths to root canal dentine. Int Endod J 2006;39:809-818.

Bitter K, Paris S, Martus P, Schartner R, Kielbassa AM. A confocal laser scanning microscope investigation of different dental adhesives bonded to root canal dentine. Int Endod J 2004;37:840-848.

Bolla M, Muller-Bolla M, Borg C, Lupi-Pegurier L, Laplanche O, Leforestier E. Root canal posts for the restoration of root filled teeth. Cochrane Database Syst Rev 2007;(1):CD004623.

Bouillaguet S, Shaw L, Barthelemy J, Krejci I, Wataha JC. Long-term sealing ability of Pulp Canal Sealer, AH-Plus, GuttaFlow and Epiphany. Int Endod J 2008;41:219-226.

Boukpessi T, Menashi S, Camoin L, Tencate JM, Goldberg M, Chaussain-Miller C. The effect of stromelysin-1 (MMP-3) on non-collagenous extracellular matrix proteins of demineralized dentin and the adhesive properties of restorative resins. Biomaterials 2008;29:4367-4373.

Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, Pashley DH, Tay FR, Mazzoni A. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater 2018;34:78-96.

Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000;1477:267-283.

Briseno Marroquin B, Wolf TG, Schurger D, Willershausen B. Thermoplastic properties of endodontic gutta-percha: a thermographic in vitro study. J Endod 2015;41:79-82.

Bueno CE, Pelegrine RA, Silveira CF, Bueno VC, Alves Vde O, Cunha RS, Pereira GD, Paulillo LA. The impact of endodontic irrigating solutions on the push-out shear bond strength of glass fiber posts luted with resin cements. Gen Dent 2016;64:26-30.

Burgin S, Rohr N, Fischer J. Assessing degradation of composite resin cements during artificial aging by Martens hardness. Head Face Med 2017;13(1):9 DOI 10.1186/s13005-017-0142-4.

Butz F, Lennon AM, Heydecke G, Strb JR. Survival rate and fracture strength of endodontically treated maxillary incisors with moderate defects restored with different post-and-core systems:an in vitro study. Int J Prosthodont 2001;14:58-64.

Cai K, Delaviz Y, Banh M, Guo Y, Santerre JP. Biodegradation of composite resin with ester linkages: identifying human salivary enzyme activity with a potential role in the esterolytic process. Dent Mater 2014;30:848-860.

Calixto LR, Bandéca MC, Clavijo V, Andrade MF, Vaz LG, Campos EA. Effect of Resin Cements System and Root Region on the Push-out Bond Strength of a Translucent Fiber Post. Oper Dent 2012;37:80-86.

Camilleri J. Mineral trioxide aggregate: present and future developments. Endodontic Topics 2015;32:31-46.

Carrilho MR, Carvalho RM, de Goes MF, di Hipolito V, Geraldeli S, Tay FR, Pashley DH, Tjäderhane L. Chlorhexidine preserves dentin bond in vitro. J Dent Res 2007;86:90-94.

Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjaderhane L, Reis, AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86(6):529-533.

Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjäderhane L, Mazzoni A, Breschi L, Foulger S, Pashley DH. Host-derived loss of dentin matrix stiffness associated with solubilization of collagen. J Biomed Mater Res B Appl Biomater 2009;90:373-380.

Carvalho JJR, Correr-sobrinho L, Correr AB, sinhoreti MA, Consani S, Sousa-Neto MD. Solubility and dimensional change after setting of root canal sealers: a proposal for smaller dimensions of test samples. J Endod 2007;33:1110-1116.

Carvalho NK, Prado MC, Senna PM, Neves AA, Souza EM, Fidel SR, Sassone LM, Silva EJ. Do smear-layer removal agents affect the push-out bond strength of calcium-silicate based endodontic sealers? Int Endod J 2017;50:612-619.

Cavenago BC, Duarte MA, Ordinola-Zapata R, Marciano MA, Carpio-Perochena AE, Bramante CM. Interfacial adaptation of an epoxy-resin sealer and a self-etch sealer to root canal dentin using the System B or the single cone technique. Brazilian Dentist Journal 2012;23:205-211.

Cecchin D, de Almeida J, Gomes B, Zaida A, Ferraz C. Effect of chlorhexidine and ethanol on the durability of the adhesion of the fiber post relined with resin composite to the root canal. J Endod 2011;37:678-683.

Cecchin D, Farina AP, Galafassi D, Barbizam JV, Corona SA, Carlini-Junior B. Influence of sodium hypochlorite and edta on the microtensile bond strength of a self-etching adhesive system. J Appl Oral Sci 2010;18:385-389.

Cecchin D, Farina AP, Giacomin M, Vidal Cde M, Carlini-Junior B, Ferraz CC. Influence of chlorhexidine application time on the bond strength between fiber posts and dentin. J Endod 2014;40:2045-2048.

Chadha R, Taneja S, Kumar M, Gupta S. An *in vitro* comparative evaluation of depth of tubular penetration of three resin-based root canal sealers. Journal of Conservative Dentistry 2012;15:18-21.

Chen WP, Chen YY, Huang SH, Lin CP. Limitations of push-out test in bond strength measurement. J Endod 2013;39:283-287.

Darvell BW, Wu RCT. "MTA" - An Hydraulic Silicate Cement: Review update and setting reaction. Dent Mater 2011;27:407-422.

Das AK, Muddugangadhar BC, Amarnath GS, Garg A, Kumar U, Rao TR. Comparative Evaluation of Push Out Bond Strength of a Fiber Post System using Four Different Resin Cements: An In-Vitro Study. J Int Oral Health 2015;7:62-67.

De Araujo DF, Chaves LP, Bim O,Jr, Pimentel Garcia FC, Ishikiriama SK, Honorio HM, Wang L. Influence of 2% chlorhexidine digluconate on bond strength of a glass-fibre post luted with resin or glass-ionomer based cement. J Dent 2014;42:735-741.

De Castro FL, de Andrade MF, Duarte Junior SL, Vaz LG, Ahid FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. J Adhes Dent 2003;5:129-138.

De Las Heras M, Valenzuela A, Overal CM. The matrix metalloproteinase gelatinase A in human dentine. Arch Oral Biol 2000;45:757-765.

De Munck J, Vargas M, Van Landuyt K, Hikita K, Lambrechts P, Van Meerbeek B. Bonding of an auto-adhesive luting material to enamel and dentin. Dent Mater 2004;20:963-971.

De Souza G, Braga RR, Cesar PF, Lopes GC. Correlation between clinical performance and degree of conversion of resin cements: a literature review. J Appl Oral Sci 2015;23:358-368.

De-Deus G, Reis C, Brandao C, Fidel S, Fidel RAS. The ability of Portland cement, MTA and MTA Bio to prevent through-and-through fluid movement in repaired furcal perforations. J Endod 2007;33:1374-1377.

De-Deus G, Reis C, Di Giorgi K, Brandao MC, Audi C, Fidel RA. Interfacial adaptation of the Epiphany self-adhesive sealer to root dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:381-386.

Dong Z, Chang J, Deng Y, Joiner A. Tricalcium silicate induced mineralization for occlusion of dentinal tubules. Aust Dent J 2011;56:175-180.

Donnermeyer D, Burklein S, Dammaschke T, Schafer E. Endodontic sealers based on calcium silicates: a systematic review. Odontology 2018:Dec 15

Duret B, Reynaud M, Duret F. "New concept of coronoradicular reconstructio:the composipost. Le Chirurgien-dentiste de France 1990;60:131-141.

El-Ma'aita AM, Qualtrough AJ, Watts DC. The effect of smear layer on the push-out bond strength of root canal calcium silicate cements. Dent Mater 2013;29:797-803.

Elnaghy AM. Influence of QMix irrigant on the micropush-out bond strength of biodentine and white mineral trioxide aggregate. J Adhes Dent 2014;16:277-283.

Erdemir A, Ari H, Gungunes H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. J Endod 2004;30:113-116.

Faria-e-Silva AL, Fabiao MM, Arias VG, Martins LRM. Activation Mode Effects on the Shear Bond Strength of Dual-cured Resin Cements. Oper Dent 2010;35:515-521.

Farina AP, Chiela H, Carlini-Junior B, Mesquita MF, Miyagaki DC, Ferraz CCR, Vidal CMP, Cecchin D. Influence of Cement Type and Relining Procedure on Push-Out Bond Strength of Fiber Posts after Cyclic Loading. J Prosthodont 2016;25:54-60.

Ferracane JL, Stansbury JW, Burke FJ. Self-adhesive resin cements - chemistry, properties and clinical considerations. J Oral Rehabil 2011;38:295-314.

Ferrari M, Mannocci F. A "one-bottle" adhesive system for bonding a fibre post into a root canala: an SEM evaluation of the post-resin interface. Int Endod J 2000;33:397-400.

Ferrari M, Vichi A, Garcia-Godoy F. Clinical evaluation of fiber-reinforced epoxy resin posts and cast post and cores. Am J Dent 2000;13:15B-18B.

Ferreira de Assis D, do Prado M, Simao RA. Evaluation of the interaction between endodontic sealers and dentin treated with different irrigant solutions. J Endod 2011;37:1550-1552.

Fisher MA, Berzins DW, Bahcall JK. An in vitro comparison of bond strength of various obturation materials to root canal dentin using a push-out test design. J Endod 2007;33:856-858.

Fokkinga WA, Kreulen CM, Vallittu PK, Creugers NH. A structured analysis of in vitro failure loads and failure modes of fiber, metal, and ceramic post-and-core systems. Int J Prosthodont 2004;17:476-482.

Frassetto A, Navarra CO, Marchesi G, turco G, di Lenarda R, Breschi L. Kinetics of polymerization and contraction stress development in self-adhesve resin cements. Dent Mater 2012;28:1032-1039.

Fredriksson M, Astbäck J, Pamenius M, Arvidson K. A retrospective study of 236 patients with teeth restored by carbon fiber-reinforced epoxy resin posts. J Prosthet Dent 1998;80:151-157.

Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the Activities of Matrix Metalloproteinases 2, 8, and 9 by Chlorhexidine. Clin Diagn Lab Immunol 1999;6:437-439.

Glazer B. Restoration of endodontically treated teeth with carbon fibre posts - a prospective study. J Can Dent Assoc 2000;66:613-618.

Goodman A, Schilder H, Aldrich W. The thermomechanical properties of gutta-percha. II. The history and molecular chemistry of gutta-percha. Oral Surg Oral Med Oral Pathol 1974;37:954-961.

Gopal S, Irodi S, Mehta D, Subramanya S, Govindaraju VK. Fracture Resistance of Endodontically Treated Roots Restored with Fiber Posts Using Different Resin Cements- An In-vitro Study. J Clin Diagn Res 2017;11:ZC52-ZC55.

Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, Tay F, Ferrari M. The adhesion between fiber posts and root canal walls:comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci 2004;Aug 112(4):353-361.

Guneser MB, Akbulut MB, Eldeniz AU. Effect of various endodontic irrigants on the push-out bond strength of biodentine and conventional root perforation repair materials. J Endod 2013;39:380-384.

Hammad M, Qualtrough A, Silikas N. Evaluation of root canal obturation: a three dimensional in vitro study. J Endod 2009;35:541-544.

Hammad M, Qualtrough A, Silikas N. Extended setting shrinkage behavior of endodontic sealers. J Endod 2008;34:90-93.

Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentine. Int Endod J 2011;44:1081-1087.

Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand 2007;65:1-13.

Hayashi M, Sugeta A, Takahashi Y, Imazato S, Ebisu S. Static and fatigue fracture resistances of pulpless teeth restored with post-cores. Dent Mater 2008;24:1178-1186.

Hayashi M, Takahashi Y, Imazato S, Ebisu S. Fracture resistance of pulpless teeth restored with post-cores and crowns. Dent Mater 2006;22:477-485.

Hinata G, Yoshiba K, Han L, Edanami N, Yoshiba N, Okiji T. Bioactivity and biomineralization ability of calcium silicate-based pulp-capping materials after subcutaneous implantation. Int Endod J 2017;50 Suppl 2:e40-e51.

Hiraishi N, Yiu C, King N, Tay F. Effect of 2% chlorhexidine on dentin microtensile bond strengths and nanoleakage of luting cements. J Dent 2009;37:440-448.

Holderegger C, Sailer I, Schuhmacher C, Schläpfer R, Hämmerle C, Fischer J. Shear bond strength of resin cements to human dentin. Dent Mater 2008;24:944-950.

Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, Rutledge RE. The Anti-microbial Effect Against Enterococcus faecalis and the Compressive Strength of Two Types of Mineral Trioxide Aggregate Mixed With Sterile Water or 2% Chlorhexidine Liquid. J Endod 2007;33:844-847.

Hong ST, Bae KS, Baek SH, Kum KY, Shon WJ, Lee W. Effects of root canal irrigants on the push-out strength and hydration behavior of accelerated mineral trioxide aggregate in its early setting phase. J Endod 2010;36:1995-1999.

Hosaka K, Nakajima M, Takahashi M, Itoh S, Ikeda M, Tagami J, Pashley DH. Relationship between mechanical properties of one-step self-etch adhesives and water sorption. Dental Materials 2010; 26:360-367.

Hosaka K, Tagami J, Nishitani Y, Yoshiyama M, Carrilho M, Tay F, Agee KA, Pashley DH. Effect of wet vs. dry testing on the mechanical properties of hydrophilic self-etching primer polymers. Eur J Oral Sci 2007;115:239-245.

Jafari F, Rahimi S, Shahi S, Jafari S. Endodontic microleakage studies: correlation among different methods, clinical relevance, and potential laboratory errors. Minerva Stomatol 2017;66:169-177.

Johnson WB. A new gutta-percha technique. J Endod 1978;4:184-188.

Kennett CN, Cox SW, Eley BM. Ultrastructural localization of cathepsin B in gingival tissue from chronic periodontitis patients. Histochem J 1997;29:727-734.

Kim J, Song YS, Min KS, Kim SH, Koh JT, Lee BN, Chang HS, Hwang IN, Oh WM, Hwang YC. Evaluation of reparative dentin formation of ProRoot MTA, Biodentine and BioAggregate using micro-CT and immunohistochemistry. Restor Dent Endod 2016;41:29-36.

Kim JR, Nosrat A, Fouad AF. Interfacial characteristics of Biodentine and MTA with dentine in simulated body fluid. J Dent 2015;43:241-247.

Kim Y, Kim S, Shin YS, Jung IY, Lee SJ. Failure of setting of mineral trioxide aggregate in the presence of fetal bovine serum and its prevention. J Endod 2012;38:536-540.

Kim YK, Grandini S, Ames JM, Gu LS, Kim SK, Gutmann JL, Tay FR. Critical review on methacrylate resin-based root canal sealers. J Endod 2010;36:383-399.

Kim YK, Mai S, Haycock JR, Kim KS, Loushine RJ, Pashley DH, Tay FR. The self-etching potential of Realseal vs RealSeal SE. J Endod 2009;**35:**1264-1269.

King PA, Setchell DJ, Rees S. Clinical evaluation of a carbon fibre reinforced carbon endodontic post. J Oral Rehabil 2003;30:785-789.

Kogan P, He J, Glickman GN, Watanable I. The effects of various additives on setting properties of MTA. J Endod 2006;32:569-572.

Koruyucu M, Topcuoglu N, Tuna EB, Ozel S, Gencay K, Kulekci G, Seymen F. An assessment of antibacterial activity of three pulp capping materials on Enterococcus faecalis by a direct contact test: An in vitro study. European Journal of Dentistry 2015;9:240-245.

Koutroulis A, Batchelor H, Kuehne SA, Cooper PR, Camilleri J. Investigation of the effect of the water to powder ratio on hydraulic cement properties. Dent Mater 2019;May 29.

Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine. J Endod 2010;36:1154-1157.

Kul E, Yeter KY, Aladag LI, Ayranci LB. Effect of different post space irrigation procedures on the bond strength of a fiber post attached with a self-adhesive resin cement. J Prosthet Dent 2016;115:601-605.

Lancaster PE. Restorative Case Report: Flexibility of Fibre-Posts. Eur J Prosthodont Restor Dent 2015;23:85-90.

Lassila LV, Tanner J, Le Bell AM, Narva K, Vallittu PK. Flexural properties of fiber reinforced root canal posts. Dent Mater 2004;20:29-36.

Le Bell A, Lassila LV, Kangasniemi I., Vallittu P. Bonding of fibre-reinforced composite post to root canal dentin. J Dent 2005;33(7):533-539.

Le Bell AM, Tanner J, Lassila LV, Kangasniemi I, Vallittu P. Bonding of composite resin luting cement to fiber-reinforced composite root canal posts. J Adhes Dent 2004;6:319-325.

Le Bell-Rönnlöf A, Lassila LV, Kangasniemi I, Vallittu PK. Load-bearing capacity of human incisor restored wwith various fiber-reinforced composite posts. Dent Mater 2011;27:e107-115.

Leitune VC, Collares FM, Werner Samuel SM. Influence of chlorhexidine application at longitudinal push-out bond strength of fiber posts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:e77-81.

Li GH, Niu LN, Zhang W, Olsen M, De-Deus G, Eid AA, Chen J, Pashley DH, Tay FR. Ability of new obturation materials to improve the seal of the root canal system: A review. Acta Biomaterialia 2014;10:1050-1063.

Marchionatti AME, Wandscher VF, Rippe MP, Kaizer OB, Valandro LF. Clinical performance and failure modes of pulpless teeth restored with posts: a systematic review. Braz Oral Res 2017;31:e64-3107BOR-2017.vol31.0064.

Maroulakos G, He J, Nagy WW. The Post-endodontic Adhesive Interface: Theoretical Perspectives and Potential Flaws. J Endod 2018;44:363-371.

Marren K. Dimethyl Sulfoxide: An effective penetration enhancer for topical administration of NSAIDs. The Phys Sportsmed 2011;39:75-82.

Martin RL, Monticelli F, Brackett WW, Loushine RJ, Rockman RA, Ferrari M, Pashley DH, Tay F. Sealing properties of mineral trioxide aggregate orthograde apical plugs and root fillings in an in vitro apexification model. J Endod 2007;33:272-275.

Massi S, Tanomaru-Filho M, Silva GF, Duarte MA, Grizzo LT, Buzalaf MA, Guerreiro-Tanomaru JM. pH, calcium ion release, and setting time of an experimental mineral trioxide aggregate-based root canal sealer. J Endod 2011;37:844-846.

Mazzoni A, Mannello F, Tay FR, Tonti GA, Papa S, Mazzotti G, Di Lenarda R, Pashley DH, Breschi L. Zymographic analysis and characterization of MMP-2 and -9 forms in human sound dentin. J Dent Res 2007;86:436-440.

Mazzoni A, Maravic T, Tezvergil-Mutluay A, Tjaderhane L, Scaffa PMC, Seseogullari-Dirihan R, Bavelloni A, Gobbi P, Pashley DH, Tay FR, Breschi L. Biochemical and immunohistochemical identification of MMP-7 in human dentin. J Dent 2018;79:90-95.

Mazzoni A, Marchesi G, Cadenaro M, Mazzotti G, Di Lenarda R, Ferrari M, Breschi L. Push-out stress for fiber posts luted using different adhesive strategies. Eur J Oral Sci 2009;117:447-453.

Mazzoni A, Nascimento FD, Carrilho M, Tersariol I, Papa V, Tjaderhane L, di Lenarda R, Tay FR, Pashley DH, Breschi L. MMP activity in the hybrid layer detected with in situ zymography. J Dent Res 2012;91:467-472.

Mazzoni A, Papa V, Nato F, Carrilho M, Tjaderhane L, Ruggeri A,Jr, Gobbi P, Mazzoni G, Tay FR, Pashley DH, Breschi L. Immunohistochemical and biochemical assay of MMP-3 in human dentine. J Dent 2011;39:231-237.

Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, Toledano M, Pashley EL, Tay FR. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials 2006;27:4470-4476.

Mazzoni A, Scaffa P, Carrilho M, Tjaderhane L, Di Lenarda R, Polimeni A, Tezvergil-Mutluay A, Tay FR, Pashley DH, Breschi L. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. J Dent Res 2013;92:82-86.

Mazzoni A, Tjaderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, Pashley DH, Breschi L. Role of dentin MMPs in caries progression and bond stability. J Dent Res 2015;94:241-251.

McLaren JD, McLaren CI, Yaman P, Bin-Shuwaish MS, Dennison JD, McDonald NJ. The effect of post type and length on the fracture resistance of endodontically treated teeth. J Prosthet Dent 2009;101:174-182.

Mehtälä P, Pashley DH, Tjaderhane L. Effect of dimethyl sulfoxide on dentin collagen. Dent Mater 2017;33:915-922.

Mehtälä P, Agee K, Breschi L, Pashley DH, Tjäderhane L. Proprietary solvent enhances wettability. Dent Mater 2010;26 (Suppl. 1):e 12-3 (Abstr. No. 25)

Miletic I, Ribaric SP, Karlovic Z, Jukic S, Bosnjak A, Anic I. Apical leakage of five root canal sealers after one year of storage. J Endod 2002;28:431-432.

Morgental RD, Vier-Pelisser FV, Oliveira SD, Antunes FC, Coqo DM, Kopper PM. Antibacterial activityof two MTA-based root canal sealers. Int Endod J 2011;44:1128-1133.

Muniz L, Mathias P. The influence of sodium hypochlorite and root canal sealers on post retention in different dentin regions. Oper Dent 2005;30:533-539.

Nascimento FD, Minciotti CL, Geraldeli S, Carrilho MR, Pashley DH, Tay FR, Nader HB, Salo T, Tjäderhane L, Tersariol L. Cysteine cathepsins in human carious dentin. J Dent Res 2011;90:506-511.

Naumann M, Blankenstein F, Kiessling S, Dietrich T. "Risk factors for failure of glass fiber-reinforced composite post restorations: a prospective observational clinical study". Eur J Oral Sci 2005;113:519-524.

Nayak G, Hasan MF. Biodentine-a novel dentinal substitute for single visit apexification. Restor Dent Endod 2014;39:120-125.

Nilsson E, Bonte E, Bayet F, Lasfargues JJ. Management of internal root resorption on permanent teeth. Int J Dent 2013:929486.

Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, Carvalho RM, Tjäderhane L, Tay FR, Pashley DH. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci 2006;114:160-166.

Ozturk B, Ozer F. Effect of NaOCl on bond strengths of bonding agents to pulp chamber lateral walls. J Endod 2004;30:362-365.

Palosaari H, Pennington CJ, Larmas M, Edwards DR, Tjaderhane L, Salo T. Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue. Eur J Oral Sci 2003;111:117-127.

Pane ES, Palamara JE, Messer HH. Critical evaluation of the push-out test for root canal filling materials. J Endod 2013;39:669-673.

Paque F, Luder HU, Sener B, Zehnder M. Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. Int Endod J 2006;39:18-25.

Pashley DH, Ciucchi B, Sano H, Carvalho RM, Russell CM. Bond strength versus dentine structure: a modelling approach. Arch Oral Biol 1995;40:1109-1118.

Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, *et al*. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1-16.

Pashley DH, Tay FR, Imazato S. How to increase the durability of resin-dentin bonds. Compend Contin Educ Dent 2011;32:60-64, 66.

Perdigao J, Gomes G, Augusto V. The effect of dowel space on the bond strengths of fiber posts. J Prosthod 2007;16:154-164.

Pereira JR, Valle AL, Ghizoni JS, Bonfante EA. The effect of post material on the characteristic strength of fatigue endodontically treated teeth. J Prosthet Dent 2014;112:1225-1230.

Pick B, Gonzaga CC, Junior WS, Kawano Y, Braga RR, Cardoso PE. Influence of curing light attenuation caused by aesthetic indirect restorative materials on resin cement polymerization. Eur J Dent 2010;4:314-323.

Poggio C, Beltrami R, Colombo M, Ceci M, Dagna A, Chiesa M. In vitro antibacterial activity of different pulp capping materials. Journal of Clinical and Experimental Dentistry 2015; 7:e584-588.

Poolthong S, Mori T, Swain MV. Determination of Elastic Modulus of Dentin by Small Spherical Diamond Indenters. Dent Mater J 2001;20:227-236.

Prado M, Ferreira de Assis D, Gomes BPEA, Simao RA. Effect of disinfectant solutions on the surface free energy and wettability of filling material. J Endod 2011;37:980-982.

Prati C, Gandolfi MG. Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dent Mater 2015;31:351-370.

Prestegaard H, Portenier I, Orstavik D, Kayaoglu G, Haapasalo M, Endal U. Antibacterial activity of various root canal sealers and root-end filling materials in dentin blocks infected ex vivo with Enterococcus faecalis. Acta Odontol Scand 2014;72:970-976.

Raina R, Loushine RJ, Weller R.N., Tay FR, Pashley DH. Evaluation of the quality of the apical seal in Resilon/Epiphany and Gutta-Percha/AH Plus-filled root canals by using a fluid filtration approach. J Endod 2007;33:944-947.

Reyes-Carmona JF, Felippe MS, Felippe WT. Biomineralization ability and interaction of mineral trioxide aggregate and white Portland cement with dentin in a phosphate-containing fluid. J Endod 2009;35:731-736.

Reyes-Carmona JF, Felippe MS, Felippe WT. The biomineralization ability of mineral trioxide aggregate and Portland cement on dentin enhances the push-out strength. J Endod 2010;36:286-291.

Roberts HW, Toth JM, Berzinx DW, Charlton DG. Mineral trioxide aggregate material use in endodontic treatment: A review of the literature. Dent Mater 2008;24:149-164.

Rodrigues RV, Sampaio CS, Pacheco RR, Pascon FM, Puppin-Rontani RM, Giannini M. Influence of adhesive cementation systems on the bond strength of relined fiber posts to root dentin. J Prosthet Dent 2017;118:493-499.

Rodriguez-Cervantes PJ, Sancho-Bru JL, Barjau-Escribano A, Forner-Navarro L, Perez-Gonzalez A, Sanchez-Marin FT. Influence of prefabricated post dimensions on restored maxillary central incisors. J Oral Rehabil 2007;34:141-152.

Rossi-Fedele G, Dogramaci EJ, Guastalli AR, Steier L, de Figueiredo JA. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. J Endod 2012;38:426-431.

Sabeti MA, Nekofar M, Motahhary P, Ghandi M, Simon JH. Healing of apical periodontitis after endodontic treatment with and without obturation in dogs. J Endod 2006;32:628-633.

Salameh Z, Sorrentino R, Ounsi HF, Goracci C, Tashkandi E, Tay FR, Ferrari M. Effect of different all-ceramic crown system on fracture resistance and failure pattern of endodontically treated maxillary premolars restored with and without glass fiber posts. J Endod 2007;33:848-851.

Santos Cardoso O, Coelho Ferreira M, Moreno Carvalho E, Campos Ferreira PV, Bauer J, Carvalho CN. Effect of root repair materials and bioactive glasses on microhardness of dentin. Iran Endod J 2018;13:337-341.

Santos J, Carrilho M, Tervahartiala T, Sorsa T, Breschi L, Mazzoni A, Pashley D, Tay F, Ferraz C, Tjäderhane L. Determination of matrix metalloproteinases in human radicular dentin. J Endod 2009;35:686-689.

Santos JN, Carrilho MR, De Goes MF, Zaia AA, Gomes BP, Souza-Filho FJ, Ferraz CC. Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. J Endod 2006;32:1088-1090.

Sarkis-Onofre R, Skupien JA, Cenci MS, Moraes RR, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. Oper Dent 2014;39:E31-44.

Sartori N, Peruchi LD, Phark JH, Lopes MM, Araujo E, Vieira LC, Belli R, Duarte S Jr. Permeation of intrinsic water into ethanol- and water-saturated, monomer-infiltrated dentin bond interfaces. Dent Mater 2015;31:1385-1395.

Scaffa PM, Breschi L, Mazzoni A, Vidal CM, Curci R, Apolonio F, Gobbi P, Pashley D, Tjäderhane L, Tersariol IL, Nascimento FD, Carrilho MR. Co-distribution of cysteine cathepsins and matrix metalloproteases in human dentin. Arch Oral Biol 2017;74:101-107.

Scaffa PM, Vidal CM, Barros N, Gesteira TF, Carmona AK, Breschi L, Pashley DH, Tjäderhane L, Tersariol IL, Nascimento FD, Carrilho MR. Chlorhexidine inhibits the activity of dental cysteine cathepsins. J Dent Res 2012;91:420-425.

Schwartz RS, Robbins JW. Post placement and restoration of endodontically treated teeth: a literature review. J Endod 2004;30:289-301.

Shafiei F, Memarpour M. Effect of chlorhexidine application on long-term shear bond strength of resin cements to dentin. Journal of Prosthodontic Research 2010;54:153-158.

Sheykhrezae MS, Meraji N, Ghanbari F, Nekoofar MH, Bolhari B, Dummer PMH. Effect of blood contamination on the compressive strength of three calcium silicate-based cements. Aust Endod J 2018;44(3):255-259.

Shibata S, Vieira LC, Baratieri LN, Fu J, Hoshika S, Matsuda Y, Sano H. Evaluation of microtensile bond strength of self-etching adhesives on normal and caries-affected dentin. Dent Mater J 2016;35:166-173.

Silva Almeida LH, Moraes RR, Morgental RD, Pappen FG. Are Premixed Calcium Silicate-based Endodontic Sealers Comparable to Conventional Materials? A Systematic Review of In Vitro Studies. J Endod 2017;43:527-535.

Skupiei JA, Sarkis-Onofre R, Cenci MS, De Moraes RR, Pereira-Cenci T. A systematic review of factors associated with the retention of glass fiber posts. Brazilian Oral Research 2015;29:1-8.

Soares CJ, Pereira CA, Pereira JC, Santana FR, do Prado CJ. Effect of chlorhexidine application on microtensile bond strength to dentin. Oper Dent 2008;33:183-188.

Soares CJ, Santana FR, Castro CG, Santos-Filho PC, Soares PV, Qian F, Armstrong SR. Finite element analysis and bond strength of a glass post to intraradicular dentin: Comparison between microtensile and push-out tests. Dent Mater 2008;24:1405-1411.

Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. Oral Diseases 2004;10:311-318.

Stape TH, Tezvergil-Mutluay A, Mutluay MM, Martins LRM, do Prado RL, Pizi ECG, Tjäderhane L. Influence of dimethyl sulfoxide used as a solvent on the physical properties and long-term dentin bonding of hydrophilic resins. J Mech Behav Biomed Mater 2016;64:220-228.

Stape TH, Tjäderhane L, Marques MR, Aguiar FH, Martins LR. Effect of dimethyl sulfoxide wet-bonding technique on hybrid layer quality and dentin bond strength. Dent Mater 2015;31:676-683.

Stape TH, Tjäderhane L, Tezvergil-Mutluay A, Yanikian CR, Szesz AL, Loguercio AD, Martins LR. Dentin bond optimization using the dimethyl sulfoxide-wet bonding strategy: A 2-year in vitro study. Dent Mater 2016;32:1472-1481.

Sulkala M, Larmas M, Sorsa T, Salo T, Tjäderhane L. The Localization of Matrix metalloproteinase-20 (MMP-20, Enamelysin) in Mature Human Teeth. J Dent Res 2002;14:603-607.

Sulkala M, Tervahartiala T, Sorsa T, Larmas M, Salo T, Tjaderhane L. Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin. Arch Oral Biol 2007;52:121-127.

Tay FR, Pashley DH, Loushine RJ, Weller RN, Monticelli F, Osorio R. Self-etching adhesives increase collagenolytic activity in radicular dentin. J Endod 2006 32:862-868.

Tay FR, Pashley DH, Rueggeberg FA, Loushine RJ, Weller RN. Calcium phosphate phase transformation produced by the interaction of the portland cement component of white mineral trioxide aggregate with a phosphate-containing fluid. J Endod 2007;33:1347-1351.

Tay FR, Sano H, Carvalho R, Pashley EL, Pashley DH. An ultrastructural study ot the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. J Adhes Dent 2000;2:83-98.

Temel UB, Van Ende A, Van Meerbeek B, Ermis RB. Bond strength and cement-tooth interfacial characterization of self-adhesive composite cements. Am J Dent 2017;30:205-211.

Tersariol IL, Geraldeli S, Minciotti CL, Nascimento FD, Paakkonen V, Martins MT, Carrilho MR, Pashley DH, Tay FR, Salo T, Tjäderhane L. Cysteine cathepsins in human dentin-pulp complex. J Endod 2010;36(3):475-481.

Tezvergil-Mutluay A, Mutluay M, Seseogullari-Dirihan R, Agee KA, Key WO, Scheffel DL, Breschi L, Mazzoni A, TjäderhaneL, Nishitani Y, Tay FR, Pashley DH. Effect of phosphoric acid on the degradation of human dentin matrix. J Dent Res 2013;92:87-91.

Tezvergil-Mutluay A, Mutluay MM, Gu LS, Zhang K, Agee KA, Carvalho RM, Manso A, Carrilho M, Tay FR, Breschi L, Sun BI, Pashley DH. The anti-MMP activity of benzalkonium chloride. J Dent 2011;39:57-64.

Thaler A, Ebert J, Petschelt A, Pelka M. Influence of tooth age and root section on root dentine dye penetration. Int Endod J 2008;41:1115-1122.

Thompson JM, Agee KA, Sidow SJ, McNally K, Lindsey K, Borke J*, Elsalanty M, Tay FR, Pashley DH.* Inhibition of Endogenous Dentin Matrix Metalloproteinases by Ethylenediaminetetaacetic Acid. J Endod 2012;38:62-65.

Tjäderhane L, Buzalaf MA, Carrilho M, Chaussain C. Matrix metalloproteinases and other matrix proteinases in relation to cariology: the era of 'dentin degradomics'. Caries Res 2015;49:193-208.

Tjäderhane L, Larjava H, Sorsa T, Uitto V-, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998;77:1622-1629.

Tjäderhane L, Mehtälä P, Scaffa P, Vidal C, Pääkkönen V, Breschi L, Hebling J, Tay FR, Nascimento FD, Pashley DH, Carrilho MR. The effect of dimethyl sulfoxide on dentin bonding and nanoleakage of etch-and-rinse adhesives. Dent Mater 2013a;29:1055-1062.

Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho M, Carvalho RM, Tay FR, Pashley DH. Strategies to prevent hydrolytic degradation of the hybrid layer-A review. Dent Mater 2013b;29:999-1011.

Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013c;29:116-135.

Toledano M, Nieto-Aguilar R, Osorio R, Campos A, Osorio E, Tay FR, Alaminos M. Differential expression of matrix metalloproteinase-2 in human coronal and radicular sound and carious dentine. J Dent 2010;38:635-640.

Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 1993;19:591-595.

Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endod 1995;21:349-353.

Turk V, Stoka V, Vasiljeva O, Renko M, Sun T, Turk B*, Turk D.* Cysteine cathepsins: from structure, function and regulation to new frontiers. Biochim Biophys Acta 2012;1824:68-88.

Turk T, Kaval ME, Sarikanat M, Hulsmann M. Effect of final irrigation procedures on fracture resistance of root filled teeth: an ex vivo study. Int Endod J 2017;50:799-804.

Ubaldini ALM, Benetti AR, Sato F, Pascotto RC, Medina Neto A, Baesso ML, Peutzfeldt A. Challenges in luting fibre posts: Adhesion to the post and to the dentine. Dent Mater 2018;34:1054-1062.

Vallittu PK. An overview of development and status of fiber-reinforced composites as dental and medical biomaterials. Acta Biomater Odontol Scand 2018;4:44-55.

Vallittu PK. Are we misusing fiber posts? Guest editorial. Dent Mater 2016;32:125-126.

Vallittu PK. High-aspect ratio fillers: fiber-reinforced composites and their anisotropic properties. Dent Mater 2015;31:1-7.

Vallittu PK. Interpenetrating polymer networks (IPNs) in dental polymers and composites**.** Journal of Adhesion Science and Technology 2009;23:961-972.

Vallittu PK, Boccaccini AR, Hupa L, Watts DC. Bioactive dental materials-Do they exist and what does bioactivity mean? Dent Mater 2018;34:693-694.

Vallittu PK, Sevelius C. Resin-bonded, glass fiber-reinforced composite fixed partial dentures: a clinical study. J Prosthet Dent 2000;84:413-418.

Vanderweele RA, Schwartz SA, Beeson TJ. Effect of blood contamination on retention characteristics of MTA when mixed with different liquids. J Endod 2006;32:421-424.

Van Landuyt KL, De Munck J, Minne A, Cardoso MV, Peumans M, Van Meerbeek B. Filler debonding & subhybrid-layer failures in self-etch adhesives. J Dent Res 2010;89:1045-1050.

Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coultinho E, Suzuki K, Lambrechts P, Van Meerbeek B. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials 2007;28:3757-3785.

Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, J. DM, K.L. VL. State of the art of self-etch adhesives. Dent Mater 2011;27:17-28.

Varela SG, Rabade LB, Lombardero PR, Sixto JM, Bahillo JD, Park SA. In vitro study of endodontic post cementation protocols that use resin cements. J Prosthet Dent 2003;89:146-153.

Vasconcelos BC, Bernardes RA, Duarte MAH, Bramante CM, Moraes IG. Apical sealing of root canal fillings performed with five different endodontic sealers: analysis by fluid filtration. J Appl Oral Sci 2011;19:324-328.

Verissimo DM, do Vale MS. Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review. J Oral Sci 2006;48:93-98.

Vidal CM, Tjaderhane L, Scaffa PM, Tersariol IL, Pashley D, Nader HB, Nascimento FD, Carrilho MR. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. J Dent Res 2014;93:269-274.

Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. Int Endod J 2012;45:42-48.

Violich DR, Chandler NP. The smear layer in endodontics - a review. Int Endod J 2010;43:2-15.

Vishnyakov A, Lyubartsev AP, Laaksonen A. Molecular Dynamics Simulations of Dimethyl Sulfoxide and Dimethyl Sulfoxide-Water Mixture. J Phys Chem A 2001;105:1702-1710.

Visse R, Nagase H. Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. Circ Res 2003;2:827-839.

Vivacqua-Gomes N, Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root fillings. Int Endod J 2002;35:791-795.

Vrochari AD, Eliades G, Hellwig E, Wrbas KT. Curing efficiency of four self-etching, self-adhesive resin cements. *Dent Mater 2009;*25:1104-1108.

Välimaa S, Perea-Lowery L, Smatt JH, Peltonen J, Budde T, Vallittu PK. Grit blasted aggregates of hydroxyl apatite functionalized calcium carbonate in occluding dentinal tubules. *Heliyon 2018;*4(12):e01049.

Watson TF, Atmeh AR, Sajini S, Cook RJ, Festy F. Present and future of glass-ionomers and calcium-silicate cements as bioactive materials in dentistry: Biophotonics-based interfacial analyses in health and disease. Dent Mater 2014;30:50-61.

Wedding J.R., Brown C.E., Legan J.J., Moore B.K., Vail M.M. An in vitro comparison of microleakage between Resilon and Gutta-Percha with a fluid filtration model. J Endod 2007;33:1447-1449.

Weston CH, Ito S, Wadgaonkar B, Pashley DH. Effects of time and concentration of sodium ascorbate on reversal of NaOCl-induced reduction in bond strengths. J Endod 2007;33:879-881.

Whitworth J. Methods of filling root canals: principles and practices. Endodontic Topics 2005;12:2-24.

Wu H, Hayashi M, Okamura K, Koytchev EV, Imazato S, Tanaka S, Tanaka Y, Sano H, Ebisu S. Effects of light penetration and smear layer removal on adhesion of post-cores to root canal dentin by self-etching adhesives. Dent Mater 2009;25:1484-1492.

Yan P, Peng B, Fan B, Fan M, Bian Z. The effect of sodium hypochlorite (5,25%), chlorhexidine (2%), and Glyde File Prep on the bond strength of MTA-dentin. J Endod 2006;32:58-60.

Örstavik D, Nordahl I, Tibballs JE. Dimensional change following setting of root canal sealer materials. Dent Mater 2001;17:512-519.